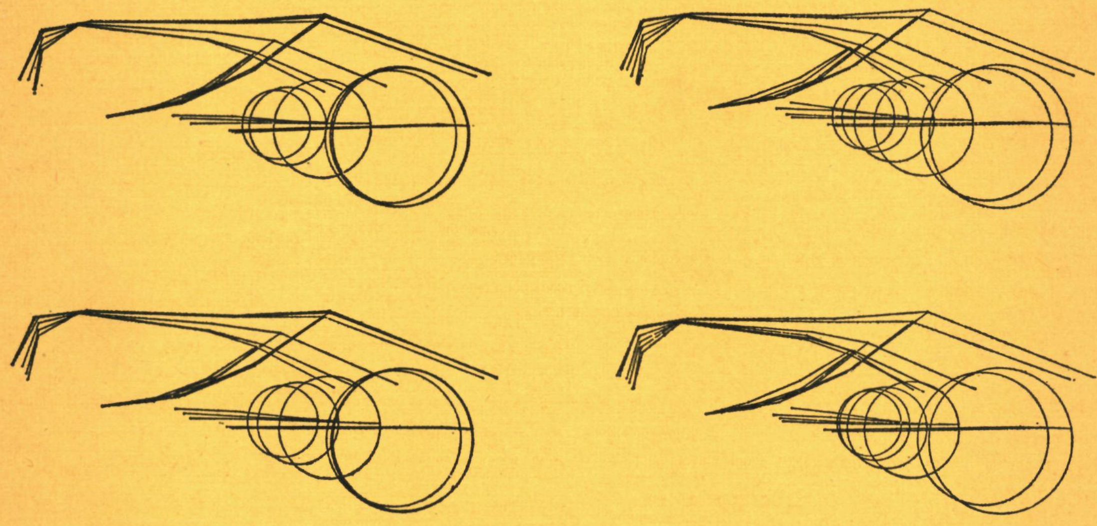
GROWTH PATTERN AND ENVIRONMENT



John F. Jefferys

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PROMOTER:

PROF. DR. F. P. G. M. VAN DER LINDEN

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DIFFERENCES IN GROWTH OF THE HARD TISSUES OF THE HEADS OF WISTAR RATS REARED IN EXTREMES OF LITTER-SIZE

A DISSERTATION SUBMITTED TO FULFIL THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF MEDICINE AT THE UNIVERSITY OF NYMEGEN,
THE NETHERLANDS, AND TO BE DEFENDED IN PUBLIC ON
FRIDAY THE 17TH OF JANUARY 1969 AT 4 P.M., ACCORDING
TO THE DECISION OF THE SENATE, AND ON THE AUTHORITY
OF THE RECTOR MAGNIFICUS MR. S. F. L. BARON VAN WIJNBERGEN,
PROFESSOR IN THE FACULTIES OF LAW AND SOCIAL SCIENCES

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BORN IN SYDNEY, AUSTRALIA

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PROEFSCHRIFT TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE GENEESKUNDE AAN DE KATHOLIEKE
UNIVERSITEIT TE NIJMEGEN, OP GEZAG VAN DE
RECTOR MAGNIFICUS MR. S. F. L. BARON VAN WIJNBERGEN,
HOOGLERAAR IN DE FACULTEITEN DER RECHTSGELEERDHEID
EN DER SOCIALE WETENSCHAPPEN, VOLGENS BESLUIT VAN
DE SENAAT IN HET OPENBAAR TE VERDEDIGEN OP
17 JANUARI 1969, DES NAMIDDAGS TE 4 UUR

DOOR

JOHN FREDERICK JEFFERYS

GEBOREN TE SYDNEY, AUSTRALIE

DRUKKERIJ GEBR. JANSSEN N.V. NIJMEGEN 1969

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PREFACE

It has been demonstrated on a number of species that environmental factors have an important influence on growth. This phenomenon has been studied by biologists, veterinarians, physicians, orthodontists, and members of other disciplines. Their interest has not been just theoretical; frequently the practical implications have been of major importance.

The work presented in the literature regarding this has usually involved a too limited number of animals and a rather short period of observation. Moreover, there are still many questions in this field to be answered and this holds particularly true for the influence of environmental factors on changes in shape, including the possibility of alterations in growth pattern and proportional relationships.

So it was felt worthwhile to study these aspects in general and the growth of the craniofacial skeleton in particular, to contribute to the knowledge and understanding of the phenomenon, growth.

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CHAPTER I

GENERAL INTRODUCTION

When two gametes join in the formation of a new organism, the genetic composition of that new individual is determined once and for all. Simultaneously it becomes dependent upon its environment for providing conditions favourable for its morphogenesis and continuing growth. The resultant of the permanent interaction between the genes and the environment in controlling the growth of the organism, traces its growth pattern.

While the complete genetic composition is present from the moment of conception, that is not to say that the total information expresses itself constantly and in the same fashion throughout the life of the individual. Part of the genetic information present plays a role only in the morphogenesis of the organism; other genetic effects may not be apparent till very late in life. The data embodied in the genes are employed on many different occasions over the entire life span of the subject, and in many combinations. For some characteristics the influence of many genes is required. On the other hand the same gene can be used in the control of several phenomena. "Somatic mutations" can confuse this picture.

For every living being the environment is a more or less constantly changing factor. A mammal such as the animals we study, is first embedded in an intra-uterine environment that normally is well adapted to its needs. After emerging from the phenomenon of birth – quite an environmental event – it will encounter the different surroundings of the new-born. But it will continue to receive the maternal care that bridges the gap between full dependence and independence. The natural environment in which it must then live will have its variations in climate, vegetation, and predators, to name but a few examples. This environment will influence what the animal can realise of the potentials that are laid down in its genetic composition.

"Genes are the prime intrinsic limits of development."* It can be seen that the growth and development of an organism primarily are controlled by a genetic pattern that determines the sequence of events and the optimum result that can be obtained. Poor environmental conditions usually result in growth or development that does not reach the standard provided for by the pattern.

* BONNER, J. T. (1952): *Morphogenesis* (page 10). Princeton N.J.: Princeton University Press.

On the other hand it may not be expected that even the most excellent circumstances will lead to a condition that is beyond that laid down in the genetic information as the maximum obtainable. Many studies have shown that the environment can have a marked, usually restrictive effect on growth, on progress toward maturity, and on form. These effects are of course more easily seen in young, growing animals. Then there are greater changes taking place; also the number of changes taking place in a relatively short time is comparatively large. In this respect too, it is clear that if the environment can disturb the co-ordination of size and time in the growth of the various systems of an individual, this may result in changes in the relationships between those systems. One has to realise that it is not the genetic composition alone, but also the timing of the functions upon which it is operating as well as the environmental conditions then prevailing that determine what the resulting effect will be. This is what makes the interaction of the genes and the environment significant. The expression of the genetic information and the environment are both more or less continuously varying in time.

The mechanism of this whole complex is somewhat obscure. In general principles, the genetic and environmental factors can be considered as acting both within the cells and on the extracellular material still within the confines of the total organism. The environment can also act entirely from outside the organism.

Morphogenesis from an amorphous blob to a complex individual involves not only change in size but attention to the morphology of the cells, of the organs and the total creature. The co-ordination of all the factors involved is of enormous intricacy, and it is not surprising that sometimes failures occur. It is quite likely that the majority of failures is due to defects in the genetic composition of the cells, since that is the site of control. Nonetheless it is also possible for environmental conditions to interfere sufficiently to produce a failure. It has been shown that many external agents can induce cleft palates in experimental animals if applied at a certain moment, though it has also been demonstrated that the incidence of cleft palates if often related to genetic factors. The teratogenic effect of drugs such as thalidomide is well known. But in general the developing embryo is so shielded from environmental disturbances that most morphological defects are likely to be of genetic origin.

The basis of genetic control is the information incorporated in the DNA chains within the cell nucleus. This regulates the enzyme systems of the remainder of the cell so as to procure the orderly sequence of events constituting the growth process. How this control is extended to the whole community of cells so as to obtain the complex organisation needed in the morphogenesis of a mammal, for example, is postulated by Monod and Jacob (1961) as an extension of their 'operon' theory. This calls for an extracellular pathway for signals. The same general idea is also a requirement in the 'template-anti-template' hypothesis of Weiss and Kavanau (1957) concerning the regulation of growth. This was adapted by Tanner (1963) in his hypothesis of the 'time tally' mechanism in the hypothalamus. Burch and Burwell (1965) have suggested that the 'time tally' is a form of auto-immunological control, and having regard to the possible importance of auto-immune reactions in aging, this may be of great significance. That there needs to be such a central control of growth in complicated organisms seems quite likely, but at present still little is known. There is no doubt however that the genes have a major influence in that overall control.

Environmental influences at the intracellular level may seem paradoxical, but it is not unreasonable to place everything outside the genes in the category of environment. Changes in the intracellular material can affect the ability of the genes to transmit their instructions. For example, the presence there of some hormones can interfere with the transfer of information from the genes (Clowes 1967). Substances such as actinomycin D can block the activity of RNA in the synthesis of proteins within the cell. Then there are, of course, the environmental factors that operate outside the cell. Nutrients must be available from the environment, and there is a need for removal of metabolites. Changes in the physical environment are of importance, such as temperature, the presence of electrical charges, and of forces such as gravity. Of all relevant components most is known of the effects of nutrition.

As is the case with most environmental influences, nutritional differences give more or less consistent effects on the many animals studied. In the rearing of farm animals many aspects have been observed in practice that have later been explained in theory. Nutritional planes of different levels have been used to produce animals of differing conformations (Henseler 1914, Hammond 1932, 1960). This is considered to be due to the way in which the growth of the bone, fat, and muscle of the animals is affected in different degrees by the standard of nutrition. It also appears that some organs such as the brain and eyes may be little affected by undernutrition (Moment 1933, McMeekan 1940). The time of sexual maturity, and the bone developmental age, are both retarded by undernutrition (Talbert and Hamilton 1955, Driezen et al 1958). Differential effects have been seen to produce changes in the form and chemical composition of long bones (Dickerson and McCance 1961, Dickerson and Widdowson 1960). The combination of such effects upon an animal will therefore lead to deviations from the growth trajectory provided for by the genetic data given at conception.

Subsequently it is observed that the return to more normal conditions is followed by a remarkable restoring force applied to the growth of the individual, that tries to correct the displacement of the normal growth process. This 'catch-up' phenomenon has been seen in animals and man (Jackson 1937, Prader et al 1963). It can be most readily seen in a study of growth velocity, when a notable acceleration can be observed in deprived animals when the inhibitory force on their growth is removed. Through that acceleration, the trajectory of growth is often displaced past the original, in the typical initial overcorrection of feedback control. It then rebounds and may settle down near the original level, or if the deprivation were in the first place of great severity, the normal situation may never be regained. Minot (1891) covered this concisely when referring to the weight of his guinea pigs: 'Any irregularity in the growth of an individual tends to be followed by an opposite compensatory irregularity.'

The control responsible for this 'catch-up' is still hypothetical, though there are theories which fit the known facts quite well. In all, the basic principle is the development of a mis-match between the regulating mechanism and the organism itself, and this corresponds in a general way with the interaction between the genes and the environment which we have been discussing.

The predominance of the genetic influence on the catch-up phenomenon is revealed by the strong relationship to it of at least one genetically determined characteristic, sex. Females are less easily displaced from their course of growth, and return more definitely to the original than do males (Jackson 1937, Tanner 1962). A further indication of the strength of the genetic components of the growth pattern was indicated in the work of Brodie (1941a, 1941b) in showing that the changes in the morphology of the skull in humans generally follow a pattern that is established at a very early age. Cases where this pattern was distorted occurred only when conditions were extreme, and that at a critical period of growth. In the last thirty years the growth and development of the cranio-facial complex have been studied in detail, and what evidence there is points to the fact that genetic factors have the greatest influence, and environmental factors appear to be of minor importance (Van der Linden 1966).

It is therefore relevant to investigate the impact of a simple environmental change on the growth of the hard tissues of the head. The way in which the growth might react to both the imposition and the removal of the change, would indicate in some way the resistance of the genetic pattern to change, and its capacity for recovery.

CHAPTER II

THE ASSESSMENT OF GROWTH

The term 'growth pattern' has no particular definition that is generally accepted. To avoid misunderstanding, a description will be given of what is meant by growth pattern in the work presented here.

Growth pattern is a broad term, and it is intended to be treated as such. In this study it is considered as a model by means of which the object it represents can be reconstructed, and since it is to represent growth it must be a dynamic model. Growth, in the sense in which we shall treat it, is the process of change in form and size of a living organism. Change is the essential feature of this phenomenon. Change not in one or two aspects, but in all the characteristics of life with the passage of time. Therefore it is inadequate to attempt to describe the pattern of growth in terms of static conditions at different times; there has to be in addition a description of how those apparently static conditions have developed from one stage to the next.

It is inevitable that observations are made at discrete intervals and that they are primarily both described and interpreted as if they represent something static. An attempt to incorporate in a model all the information needed for a more complete description of the phenomena of growth leads to considerable lack of comprehensibility. In practice it seems hardly possible to build a tangible model that would show how these static attributes have been linked together by the dynamics of growth. However it is at least theoretically possible to construct a mathematical model (Medawar 1950, Walnut 1967). That is not to say that such a model will be any more comprehensible to ordinary people than is the form of simultaneous expression of several variables which is presented in some forms of modern art.

Although the process of growth may be complex in the way in which it is as a rule contributed to by a great number of factors, and although the description of the results may be difficult, it is as a concept, simple. When something grows, its dimensions change. When the same measurement of a growing object is taken twice with an interval between the two recordings, a simple change can as a rule be found. Unfortunately it is more complicated when one wishes to follow the growth of a living organism in detail and over a longer period. Not only is there a change in most dimensions; frequently the relationship between the dimensions changes also, resulting in a noticeable change in form.

Shape cannot be expressed in terms of a measurement. However, an acceptable approach is to observe the behaviour of as large a set of points as possible on as many occasions as possible, and by empirical means attempt to find a mathematical expression of their behaviour. Nevertheless to date no completely satisfactory expression has been found for even one dimension of a complex growing organism. This provides no encouragement to explore the possibility of finding a function to fit shape changes. Indeed, as F. Yates (1950) remarks, it is highly likely that the process of evolution has provided 'a complicated jumble of detailed controls superimposed on one another, conditioned in large part by historic evolutionary development rather than a simple and orderly set of controls such as might be arrived at by designing the organism completely afresh to a given specification. The embryonic development of human beings, for example, provides striking illustration of how persistent these historical factors can be.'* From the above considerations, it will be clear that there still exist many problems regarding the mathematical analysis of the physical laws underlying growth, despite its convenience as a descriptive method.

Ideally one would prefer to investigate the pattern of growth in its three-dimensional entirety. More practicable, is the investigation of the growth in just one plane. The technique of integrating three-dimensional measurements of growth changes is not yet sufficiently developed to make possible a contribution by this method that adequately would reward the effort involved. Furthermore, an acceptable exposition of the results of such work presents considerable problems.

On the basis of these remarks it will be understandable that the study of linear growth incorporated in this thesis is mainly limited to one plane of space. For example, for the rat skull the changes in a set of points pertaining to the structures in or about the mid-sagittal plane are investigated. These points determine the type of growth pattern displayed in the cephalometric part of this work. It includes a description of the behaviour of the several points in their relationship to each other, expressed as a function of time. The treatment of the descriptions is aimed at clarifying the relationships, both regarding size at one time and regarding the way in which the changes have occurred between one time and another.

Growth curves have been used to illustrate how one character of an organism has changed in time. The successs of this technique in extending the information

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* YATES, F. (1950): *The Place of Statistics in the study of Growth and Form* (page 487). Proc. Roy. Soc. B 137: 479–488.

obtained into the realms of underlying factors in growth has not been as great as its exponents might wish. But as a means of description, curves are usually very effective.

In principle, all types of curves can be expressed by mathematical functions, and it is relatively simple to find a formula that will provide a curve to fit almost any form of growth. This is particularly true since the development of computers.

The application of a growth curve to data obtained from sequential measurements of a dimension, is most useful in obtaining estimates of velocity, acceleration, and ultimate size. This last warrants cautious use, as does all extrapolation. The estimation of velocity and acceleration that is possible by differentiating the function of the growth curve at any point in time, is somewhat better, though it is nonetheless susceptible to the choice of the function used.

It is fitting at this point to emphasize that estimation always remains estimation. In general the precision of an estimate is mainly dependent on the quality of the information provided. When a measurement can be made in place of an estimate, the degree of uncertainty will always be less if the same degree of care is employed in all measuring, and the information as to the behaviour of the character in question is adequate in both cases. A corresponding situation exists here as in comparing the difference in information provided by the measurements of a collection of individuals, and by the statistics that give an estimate of the properties of those measurements. Taken per individual the measurements give no indication of the character of the other individuals, but they present a precise indication of their own character. When all are taken together, they give valuable information regarding the population from which they are a sample, but the degree of uncertainty regarding the individual measurements is now considerable.

Because consecutive observations of individuals provide additional information, incorporating more of the characteristics of individuals into the description of the population than does material not followed individually in time, it is important to take advantage of that type of study, where possible. The features of these studies, usually referred to as longitudinal investigations, are later on dealt with more fully.

With the limitations previously outlined in mind, the problem now becomes that of assembling the information that has been collected, so as to display how the individual patterns of growth combine in the overall pattern to be seen particularly in the growth of the structures making up the mid-sagittal plane of the rat skull, and how that pattern differs between the experimental groups. If that information is partitioned into a series of simple phases in the growth of the animals being studied, and combined with the accessory information on other growth aspects, a pattern may be created that still permits the observer to see how the overall picture is painted.

The form of partitioning we shall employ is to describe the changes in various characteristics – e.g. weight, size, proportions – seen in the experiments both between the experimental groups at one time, and within the same group at different times. Individuals can supply particular information about changes with time, and these changes must also appear in the descriptions we give. In a general way, it will also be interesting to see how different experimental changes will affect the growth pattern.

Three forms of presentation of our findings have, in the main, been used. In tables most general trends and details are given. Condensation of this information permitted publishing a wider coverage of the data. Graphs are used as an effective illustration of particular aspects of growth pattern. They are plotted and laid out in such a way as to make meaningful comparisons with other graphs readily possible. In addition, outlines of 'mean rat' skulls are provided to make changes in form and size more easily recogniseable. Almost all these graphs and skull outlines were made by computer, the I.B.M. 360/50.

In this chapter some aspects of the assessment of growth have been discussed; special attention has been paid to growth pattern. The difficulty of presenting such a model on paper has been explained and some of the forms in which we shall present the material have been outlined. The importance of serial observations on individuals has been introduced, and will be further developed in a following chapter.

CHAPTER III

THE NATURE OF THE EXPERIMENTS

CHOICE OF ENVIRONMENTAL FACTOR

There are many ways in which the effect of environmental factors on growth pattern can be investigated. It was felt that the most suitable approach would be to take two samples of young animals out of a uniform population. They should then be subjected to a difference in environments as sole variable that, although simple, would produce a readily measureable difference between the samples. Such a situation could be provided under several circumstances. While the mechanisms by which they are achieved are by no means fully understood, there is no doubt that some changes in growth can be produced. Those conditions most likely to be useful in this respect could be divided broadly into:

Alteration of the plane of nutrition
Acute infection or acute starvation
Alteration of the physical environment, e.g. in temperature
Administration of drugs, hormones or other media
Birth-size regulation.

These conditions, except of course the last, could be imposed at any age, but have generally been used after weaning. They have to be considered with respect to their usefulness and convenience in producing the differences desired. Some aspects of the methods indicated above will be discussed and it will be outlined why a certain approach was preferred.

Alteration of the plane of nutrition

Direct changes in the plane of nutrition have frequently been used in the study of older animals (Hatai 1907, Osborne and Mendel 1914, Jackson 1925, McCay et al 1939, McMeekan 1940). It involves careful control of the food consumed by the animals over the required period. Food restriction has been seen to reduce the growth of the experimental animals to a degree depending on the severity of the change. Generally an attempt has been made to supply all essential nutrients but at the lowest convenient level. Much important work has been done by this technique, in studying the effects of specific constituents of the diet. Not only has deprivation been used; enhancement of the supply and quality of the food has been of importance (Osborne and Mendel 1926).

In the sphere of agriculture, the impact of regulation of the plane of nutrition to secure good conversion rates from feed to stock has been considerable (Brody 1945). McMeekan's work (1940) has shown how the form and body composition of animals can also be regulated by manipulation of the plane of nutrition. More extraordinary effects on form have been shown by McCance (1960). He showed that very severe restriction in pigs can lead to a major change in the proportion of the head to the body.

Above all, the observation of the capacity of animals to recover after severe repression of their growth has been of great interest. However, experiments maintaining a very low plane of nutrition have been hampered by a high mortality rate. Such experimental work makes considerable demands on the persons who are responsible for the care of the animals and is difficult to keep under control.

An indirect way to affect the plane of nutrition is to vary the size of the litter in multiparous animals. This effect has frequently been noticed but seldom used in growth studies. Limitation of litter-size has been used by animal breeders for a very long time to obtain optimum conditions for their animals. In the reverse sense, the technique of creating very large litters so that the resources of the mother are stretched beyond their limits has been applied successfully for scientific purposes (Kennedy 1957a, Widdowson and McCance 1960). There seem to be few disadvantages in this technique, the principal one being that there is no control of the nutrition of individual members of a litter. This could lead to variations due primarily not to nutrition but to inability to meet physical competition.

Other approaches to affecting growth while the animals are being fed by the mother could be by imposing conditions that would affect the milk supply. Supply can be enhanced by ensuring that the mammae are stimulated by complete emptying, and can be reduced by preventing that emptying (Hammond 1936). Many forms of stress on the mother will reduce lactation. Surgical reduction in the number of mammae is an approach that is promising, as it might make it possible to have all conditions for the litters similar except the milk supply. A restricted milk supply can also be obtained by separating the animals from the mother for part of each day (Jackson & Stewart 1920, Eayrs & Horn 1955).

The use of one standard size of litter and controlling the milk supply by restricting the number of mammae may have certain advantages over varying the litter-size only. However in our opinion the latter is to be preferred, because avoidance of operative procedures is felt to be an essential advantage and furthermore other investigators have already shown that litter-size control can be a successful method.

Acute infection or acute starvation

The approach of acute infliction of infection or starvation is in itself interesting because in some circumstances a short episode nonetheless makes a permanent mark on the growth of an animal (Acheson and MacIntyre 1958). It reduces the necessity for prolonged care of the animal on a maintenance diet, but again there is a rather heavy mortality due to the severity of the impact needed to secure a convenient degree of change.

Alteration of the physical environment, e.g. in temperature

Changes in the physical environment such as temperature changes have been used to affect growth. Relatively simple changes can produce significant effects (Sumner 1915, Scow 1944, Roubicek 1966). Practically any situation producing stress, such as noise, crowding, aggression, will affect the growth of young animals (Diamond et al 1965). The interpretation of effects may be difficult due to the complexities possible in stress situations. Heat or cold though, have a more direct effect on metabolism and could well be used for this work. However, a clear restriction is that the effect might not be felt at a very young age if maternal care was good. Certainly in the case of low temperatures the mother would normally provide effective counter-measures.

Administration of drugs, hormones or other media

Interference by means of drugs and hormones has been used principally to study the action of various biological mechanisms (e.g. Becks et al 1948). It is said to be a useful means of modifying growth but for our purposes it suffers from a too specific action. It is often difficult if not impossible, to determine the mode of action of the agent. For example, growth changes may be seen after administering ACTH to young rats (Moss 1955, Johannessen 1965). But then not only is the complex action of ACTH on the endocrine system, as a whole, in evidence; there is reduced intake of food as well. Under these experimental conditions there is not only a generalized effect, but one or more specific ones. Theoretically this type of difficulty is only solved by an extensive investigation of the mode of action of the material used supposing that this should produce all the information needed for its proper interpretation. In a study of overall growth it would then be the aim to use those agents whose action is expected to be of a general nature only. On the basis of the considerations given here, it will be clear that this method is consequently put outside the range of our project.

Birth size regulation

In animals where multiple foetuses do not lead to shorter gestation, newborn animals are smaller as the number in the birth increases (Gates 1924, Parkes 1926). Reduction in size in utero can be attributed to the maternal services available, these principally being accomodation and nutrition. The nutritional status of the mother is important and can affect the size of her offspring (Wallace 1948, Paynter & Grainger 1956). The size of a hybrid is generally more influenced by the size of a small mother where birth size is concerned (Walton and Hammond, 1938) though subsequently it may accelerate in growth in order to reach a size more in keeping with its sire's dimensions if that is indeed marked out on its hereditary pattern. While there is obviously a possible use for these methods in procuring differences in animals at birth, unknowns were felt to be too considerable. There is a restricted choice of animals in which the effect can be found, and the advantage of an early change might be nullified by the difficulty of determining the characteristics, before the change was imposed, of the population from which the animals were drawn.

It will be clear from the information presented above that the first method described – the alteration of the plane of nutrition – was the one best suited to our purpose. In this, preference was given to the use of extremes of litter-size.

MANNER OF APPLICATION OF CONDITIONS

It can be said that, on the whole, the permanence of the effect of the conditions discussed above is dependent on:

Severity of the treatment
Time of application
Duration of application
Type of animal
Sex of the subject

These were the next factors to be considered in the design of the experiments, and will be discussed in some detail as they motivate the approach used in this study and form part of its foundation.

Severity of the treatment

It has been known for centuries that the severity of the conditions imposed on the nutrition of an animal will influence the extent of the consequent growth change (McCance 1962). In deciding on the experimental conditions the mortality of the animals is the primary consideration. Less severe methods are sought, and manipulation of the litter-size has evidently produced adequate changes with a favourable mortality rate.

Time of application

It has been suggested that the consequences of altering growth may differ according to the developmental stage of the animal when the change is made (Widdowson et al 1960). This can be due to a change in the rate of growth that does not conform with the change in the rate of maturation.

In experimental animals it has been shown that under conditions where growth was retarded, the progress of maturation was not retarded to the same degree (Acheson & MacIntyre 1958, Dickerson & Widdowson 1960). This seems to correspond with findings in human subjects who have suffered severe disturbances such as illness or famine (Acheson & Hewitt 1954). Some animals may recover entirely from the depression of their growth, but in other cases this is not so. In male rats growth restriction led to permanent dwarfing unless the restriction was imposed after the age of 9 weeks (Widdowson & McCance 1963). Where the recovery has only been partial, the composition of the animals is found to have a less mature form than in normal animals, both physically and chemically (Dickerson & Widdowson 1960). This does not apply to all parts uniformly, but indicates a 'priority of growth' in that the earlier developing parts are less affected. Similar results have been found in pigs (McMeekan 1940, McCance et al 1961).

In normal growth it can be seen that various systems differ in the way in which they approach maturity (Scammon 1930). The brain grows rapidly in the first years of life so that in man it is near its mature size at the age of 6 years. The lympoid tissue also grows rapidly but at adolescence begins to regress so that it is only about half its previous maximum size by the time adulthood is reached. Even within the one system, for instance the skeleton, the final size of the individual parts is achieved at different times (Tanner 1962).

It is therefore not so surprising that the co-ordination of growth rate and rate of maturation is upset by experimental procedures, but that despite this there is a mechanism whereby in some circumstances the discrepancy can be corrected.

In seeking to gain some insight into this phenomenon, in particular with respect to the growth of the hard tissues of the head, it is apparent that any condition that is to register an effect on the teeth must be imposed while they are still forming. This generally will require imposition at birth, though the continuously forming rodent incisor offers more latitude. At the same time, it is also important to have the conditions operative while the bones of the skull are growing and before they fuse, though it could be interesting to observe what happens if some of the bones are completed before the restrictions are imposed so that the growth of the incomplete bones alone is affected. The possibility of differing effects being seen between the cranial and facial structures would depend on the presence of such a 'priority' as that mentioned above.

This leads to the establishment of one requirement for the experimental method: that it must provide for imposition of the conditions at a time very close to the time of birth.

Duration of application

Having decided on the desirability of imposing restrictions at a time close to birth, it seemed convenient to use the natural weaning time for the determination of the duration of the intervention. A longer duration seemed unnecessary in view of the results of other workers, as the changes procured so early in life are of significant magnitude even after a comparatively short period of intervention.

Type of animal

The animals to be considered are logically the normal laboratory animals.

Primates would probably give no more information in this type of basic investigation, than would animals that grow faster and are less troublesome. Larger animals such as pigs also grow slowly and demand much space. Multiparous animals are essential for application of nutritional control by means of litter-size. Mice are rather small for ease of measurement. Rats are known to provide good results in litter-size experiments (Kennedy 1957, Widdowson & McCance 1960). Rats have several attributes useful for this study:

Birth occurs at an early stage of development, making the animals accessible for environmental effects that could only be obtained in utero in many other types of animals

Development of the second and third molars and probably also the first, could be influenced by conditions imposed at birth

The dentition consists not only of permanent molars that erupt early in life, but there are continuously erupting incisors

The development of the calvaria is almost complete at the postnatal age of 28 days when it is the practice to wean rats in the Central Animal Laboratory of the University of Nymegen, where they would be housed

The skull of the rat is of typical mammalian type, and is of the thin-walled variety analogous to the human

There is considerable information available on their growth

They grow quickly enough to make both breeding and a longitudinal study feasible

As laboratory animals they are very well standardised, and their care is no problem as a rule

The size of the animals in convenient

They are easily handled

They are relatively inexpensive.

Sex of the subject

It was desirable to use animals of only one sex, to avoid the necessity for later eliminating any experimental effects due to that factor. Of the two sexes, it is evident that the male is more susceptible to adverse circumstances, and that his absolute growth is eventually greater than that of the female (Jackson 1937, Acheson & MacIntyre 1958). Since we required as pronounced a change as possible as a result of the experiment, the male sex was indicated.

In conclusion, it was decided that the preferable method would be litter-size manipulation using male rats. The difference in litter-size would be imposed at birth or very soon afterwards. This would involve a group made up of several litters comprised of only three rats, and a parallel group of litters each containing 15–18 rats. The difference between the experimental groups would be maintained till weaning and then eliminated. With suitable precautions it was felt that the animals could be expected, at the inception of the experiment, to be similar samples out of the same population. The intervention itself is considered to be not far from being a natural phenomenon, and it is probably the least difficult to apply.

In most experimental procedures a control group of animals is used for comparison. This was not the case in our study. It was felt to be of more value to use two experimental groups in conditions of extremes. While it can be argued that there can be no better control of growth than the optimum, this is not the point. Where only the difference between the two groups is being investigated, a control is considered to be superfluous. The advantage of this approach is that the procedure provides a larger difference to be measured than would be the case if measurements were made to a control that would lie somewhere in the middle between the two extremes.

As litter-size manipulation has seldom been used in growth studies, the general basis of the method will be described with reference to some of the work in which it has been investigated.

While it has been apparent to breeders that the size of their animals was favourably influenced by the restriction of litter-size, it has also been seen that the effect is most obvious at the time of weaning. It has been suggested that final size depends not so much on weaning size as on the overall duration of growth, and its rate (Castle 1922).

It has since been shown that animals retarded some time after weaning can indeed regain their original trajectory and reach the same final size as normal controls. A restriction imposed earlier in life usually does not permit this recovery, so that the differences existing between the conditions in big and small litters are able to make a permanent impression on the growth patterns of the members of those litters (Widdowson & McCance 1963). The mechanism by which this occurs is not understood, though the concept of Tanner (1963) seems to be in agreement with the facts. He suggests that growth activity may depend on a mismatch between an inhibiting agent produced proportionately to the increase in material in the body cells, and a 'time tally' provided by maturation of intracellular material in certain nerve cells, probably in the hypothalamus. He believes that this mismatch may be permanently reduced in the circumstances of undernutrition early in life, by modification of the 'time tally' cells.

Remarkably enough, this corresponds in some ways with the observation of Kennedy (1957a) that in these same conditions of litter size differences, the fast or slow growth rates were established within the first week after birth. Simultaneously he noticed that the appetites of the rats were permanently set at levels corresponding to their future growth curves. This can also be seen to be the case for instance in the work of Jackson (1937) where he showed that on refeeding, his test animals consistently ate considerably less than normal animals, though in this case the animals had been starved after weaning, and not before. Here the hypothalamus may again be important since lesions in the hypothalamus have been shown to affect appetite very profoundly (Kennedy 1957b).

Work on the relationship between birth weight, litter-size and weaning weight clearly established a connection between all three in mice (Parkes 1926). Both birth weight and weaning weight were inversely proportional to the litter-size, and the increment in weight during weaning was also in proportion to the birth weight.

The influence of these factors beyond weaning age has also been shown to persist (Carmon et al 1963). The effect decreases in relative importance with age. Where the effect is made more obvious by the use of extremes of litter-size, the differences can be seen to persist permanently (Widdowson & McCance 1963).

It is assumed that the differences produced in these circumstances are due primarily to the extent to which the nutrition of the litters is affected. This proposition is supported by experiments by which the milk supply of nursing animals has been related to the litter-size.

Maternal milk is understandably assumed to be a satisfactory source of nutriment for the young. But an inadequate quantity could be a cause of reduced growth. By fostering young mice out to rat mothers, whose supply of milk would be more than adequate for litters which would have from their number imposed a severe drain on the resources of a mouse mother, it was possible to see that the size of the litter had little bearing on the growth as long as the food supply was generous (Parkes 1929). Nevertheless even in those conditions it seems from these data that there still was a litter-size effect.

The effect of litter-size has been eliminated in work in which standard litters have been used, so that other factors could be examined that have a bearing on the quantity of milk provided. The number of active mammary glands may vary between 8 and 12 with litters of 6 rats, and the total weight of the litters is related to the number of active glands (Dikshit & Taskar 1956). Radiation of mouse mothers with varying doses of x-rays shortly after parturition led to reduction of growth of the young, compared to mice in normal litters of the same size. This was accounted for by reduced lactation (R. Rugh 1956).

Stimulation of the mammae to lactate is connected with their being emptied (Brody 1945, Hammond 1936), so that it is possible to visualise a large litter as automatically increasing the stimulus to milk production. The capacity of the mother to fill the demand would not therefore be directly taxed till the litter-size was increased beyond a certain level. Nor would a small litter-size per se bring with it a greater milk supply per individual. This would depend rather on the appetite of the members of the litter. Reducing litter-size to five at birth, then in steps to a litter of one mouse only at five days after birth, resulted in a better growth rate than when the litter was reduced to one animal directly after birth (MacDowell et al 1930). This seems to confirm the importance of stimulation.

A further influence on the quantity of milk available would be the breeding of the mother. This has an everyday application to dairy cattle, where milk yields vary enormously between breeds. MacDowell et al (1930) showed this also in mice, and it is also a common thing to see obvious differences between individual mothers of the same strain. The same workers, by fostering experiments, have illustrated this by being able to display the fluctuations of weight of the litter occurring with the same frequency as the foster-mothers were alternated.

It is unlikely that nutrition is the only element involved in changing the growth pattern of rats in various sizes of litter. Stress effects can be expected to have an impact in several ways. The imposition of a large litter, larger than the number of mammary glands for example, would strain both the young and the mother. The young would be suffering from heavy competition. They would, if small, be in an increasingly difficult situation, since their smallness would make them less successful in the fight for food. The smaller animals would be also in a relatively more unfavourable condition from the point of view of their needs for body temperature maintenance, since their surface area would be greater in proportion to their weight. It is possible to imagine that the reaction to chronic stress would be an alteration in the hormone balance that would adversely affect the growth of the animals (A. Hatch et al 1963).

The stress effect on the mother is characterized by an agitated demeanour not seen in mothers with small litters, and the standard of care is notably lower. The young in large litters are dirty and unkempt. The animals in small litters are sleek and glossy (Plate II). Stress has a pronounced effect on lactation, and thus will exert direct effect on nutrition by reducing the milk available (Hammond 1936).

The application of litter-size manipulation has been seen in the work of Kennedy (1957) and Widdowson & McCance (1960). The use of the examination of differences between the two extremes of litter-size was made by Kennedy (1957b) and Kennedy and Pearce (1958). Following this work, Widdowson and McCance who sought among other things to investigate the effect of small size without superimposed chronic undernutrition, made extensive use of the approach.

It is naturally important when one is studying growth phenomena, to know if the experiment has imposed pathological conditions on the animal being studied. In this connection, Widdowson and McCance (1960) stated that 'both large and small animals were to all outward appearances equally normal and healthy. The small ones were not in any sense abnormal, nor did they die, as severely undernourished animals so often have done in previous investigations'.* Widdowson and Kennedy (1962) were satisfied that the manoeuvre with litter-size enabled them to eliminate the traumatic effects of chronic undernutrition

* 1960. Proc. Roy. Soc. B. 152: page 189.

which they felt masked the results of earlier work (McCay et al 1935, McCay et al 1939). Other workers using a different approach to growth restriction which involved undernutrition of the mothers in experiments using rats, found difficulty in seeing abnormalities in the young even where clear deficiencies existed in the mother's diet (Paynter and Grainger 1956). This was despite the fact that the growth of the young had been significantly reduced as a result of the regime imposed on the mother. This is felt to support the proposition that though reduced in quantity, the diet in large litters, because of its 'ideal' composition is even less likely to lead to any pathological changes.

This chapter has embraced the requirements of the experiments, and the means by which they could be satisfied. The method preferred was manipulation of litter-size in rats. Only males would be used, and the effect of the conditions in the litters would be expected to be to produce changes in the growth of the rats even though they would be fed ad libitum upon weaning.

The basis for expecting the method to be effective was outlined, supported by the experience of those who have employed it previously.

It was also indicated that the purpose of the experiment was to utilize differences in environment and to examine the consequent differences in growth. It was not the intention to make a comparison with normally growing animals.

In the next chapter the details of the method will be given, and ancillary techniques described.

CHAPTER IV

MATERIAL AND METHOD

The indivudual details of each experiment will not be presented in this chapter, but in the ones where the work itself is described and discussed. In this chapter information will mainly be given regarding the animals used and the way in which they were treated. This will be gone into fairly thoroughly since it is felt essential to present the experimental conditions in detail in a study where an investigation of the effect of one particular environmental factor was undertaken.

The type of records used in the experiments will be mentioned and a description given of the method of obtaining weights and cephalometric records, since these two records are common to all series.

The animals

The experimental animals were Wistar albino rats originating from the T.N.O.* Animal Breeding Centre in Zeist, the Netherlands. These rats were brought from the Wistar Institute to England in 1927, where they were kept as an inbred colony. Then in 1933 a number were imported from that colony to Amsterdam and eventually to Zeist in 1958. The closed colony system of breeding was used from 1941.

The original rats used in this work were bred from animals obtained directly from T.N.O. Later, all animals used for breeding had been born in the Central Animal Laboratory of the University of Nymegen (Head: Dr. Vet. M. J. Dobbelaar). The animals were of remarkable uniformity. Birth weight was 5.5 gm with a standard deviation of 0.4 gm. All the 59 litters used in this work were born on the 23rd day after conception. The number in the litters was generally 9–12.

Rearing the Animals

The experimental animals were bred by placing groups of 5 or 6 females with a male overnight. Sufficient groups were employed to expect 15–20 females

* De Centrale Organisatie voor Toegepast-natuurwetenschappelijk Onderzoek (T.N.O.). The Central Organisation for Applied Scientific Research in the Netherlands.

to have mated by the morning, when the males were removed. Vaginal smears were taken and the animals that were positive were caged apart from the other animals. 15 Days after conception they were transferred to the nursery cages in the experimental area, where they remained in separate cages thenceforth. On the morning of the 23rd day after conception most of the litters had been or were being produced. This procedure eliminated any possible difference in the samples that could have been due to lack of correspondence in ages or to temporal differences in environment. Henceforth all ages will be given *from the time of conception*, with post-natal age added where pertinent.

Allocation to Experimental Groups

The further treatment of the litters was designed to remove the effect of possible differences due to heredity or intra-uterine conditions in the experimental groups to be formed.

The rats were sexed at birth by comparison of the genito-anal distance. The females were discarded, and the number of males per litter made even, by discarding if necessary.

The males were then numbered by toe amputation (Reitsma 1963) and weighed. The numbers given were in the chronological order in which the animals were treated.

At this point the rats with even numbers were put in one box and the odd in another. These boxes were lined with cellulose wadding and warmed by a light bulb close above them. The odd and even numbered rats were accumulated in these till the total required for the experiment was reached.

The young were now redistributed to the mothers according to a random number table. It was thus completely due to chance if a young rat was with his own mother, or in the large or small litters, or with a good or a bad mother. Due to the splitting into odd and even numbered groups, each rat in one experimental group had a partner in the other group. All odd numbers belonged to one experimental group, the even to the other.

All further redistribution of the rats, as at weaning, and in reversing the conditions at 30 days post conception in one series, was done by random number tables drawn up beforehand.

Fostering

As is well known, rat mothers employ cannibalism as the best solution to family problems! With this in mind some pilot studies were made on the way in which rats accepted fostering. There turned out to be little difficulty, and indeed mothers with small litters were apparently just as likely to eat their young as were those with more responsibilities. So no special precautions were taken to ensure acceptance of the young.

Food

The rats and their mothers were fed with Hope Farms rat biscuit*. In the nursery cages this was available in a hopper attached to the cage wall so that the young had access to solid food as soon as they wanted to reach it.

Upon weaning, the rats were given free access to food 24 hours a day. Water had always been available ad libitum. No additional items of diet were provided with the rat biscuit as all nutritional requirements seem from long experience to be satisfied by its constituents.

Post-weaning Conditions

Weaning was done on the 51st day after conception by merely removing the young from the mother's cage and redistributing them according to a random number table to new cages. In each of the new cages four animals were placed, and they remained in the same cages till sacrifice.

Accomodation

The Central Animal Laboratory of the University of Nymegen is a modern building designed for optimal conditions for animal care. Ventilation is entirely via an air-conditioning system which maintains the general environment at 23–25 °C with a relative humidity of about 50%. Every room has natural light from windows at one end, but each day throughout the year the rooms are illuminated by fluorescent lighting in addition, from 8.30 a.m. till 6 p.m.

The cages were of galvanised steel. Those used for the nursery room were approximately 43 × 19 × 13 cm and were provided with peat and wood-wool for nesting. They provided a sheltered environment. The cages used after weaning were of open mesh with a raised floor, and measured about 35 × 29 × 15 cm.

Attention to the animals was by well trained, enthusiastic staff. It was regular and conscientious. There was rarely a sign of disease in the animals. All deaths occurred either in the litter, when animals were eaten by the mother, or were accidental due to the anaesthesia used in taking some of the records.

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* Hope Farms Standard Laboratory Diet R.M.H.-B, Hope Farms, Leiden, Netherlands.

Records

In this study several types of records were taken, and where possible were repeated to obtain longitudinal information. Eruption-time differences were of considerable interest but as their investigation demands very critical experimental design (Bodegom 1969) this was not proceeded with after a preliminary skirmish. The records used were:

Details of litters as born

Details of redistribution of the litters

Observation of external features such as opening of eyes, growth of ears, damage to tail, etc.

Black and white photographs of some of the animals

Tail and body lengths

Cephalometric x-ray pictures from lateral and dorso-ventral directions.

After sacrifice further records were taken of:

Cranial capacity

Weight of adrenal glands

Mandibular molar size

Histological appearance of relevant tissues.

The purpose of the records taken of the living animals was the study of their growth. The records taken after death were to obtain an impression of the correlation between the collected longitudinal information and the end result.

Of the methods of record taking, only details of weighing and making cephalometric x-ray pictures will be given here.

Weighing

Weighing was performed at about the same time in the morning on each occasion. It was carried out with a Berkel type E balance, with scale divisions in half grams. The frequency of weighing was allowed to fall off as the animals aged, since significant changes no longer can be discerned over shorter periods.

Cephalometric x-ray Records

These were taken using a standardised technique. A cephalostat for rats was employed, which provided positive fixation of the head by ear-posts. Two x-ray tubes were used so as to make it possible to take pictures of the head in two planes without altering anything in the set-up (Plates I and III). The tubes were mounted in fixed relation to the cephalostat, with the focus-film distance 700 mm. The central rays of both tubes were directed perpendicular to the films and aimed at a point 15 mm anterior to the ear-posts. The cephalostat and the filmholders formed one unit and the operating mechanism for the earposts worked symmetrically about the sagittal plane. This made it possible to maintain a constant distance of 24 mm between the midsagittal plane and the respective film. The relationship between the transporionic axis and the horizontal film was also maintained at a constant 24 mm.

The body of the rat was supported by a platform which could be adjusted vertically independently of the cephalostat. In the case of newborn and week old rats, the ear-posts were not used, but the rats were laid on their backs in a cradle located by the ear-posts. Their heads were allowed to droop over the edge of the cradle in the region of the central ray. Orientation of the heads was then by eye, guided by the nasal pin of the instrument.

For the sagittal exposures a Philips Super-Practix x-ray set was used, at 72 KVp, 20 MA, for 0.3 seconds. The dorso-ventral exposures were taken with a Philips Practix, at 65 KVp, 20 MA, for 0.5 seconds. The target of these tubes is $1.8\ mm \times 1.8\ mm$.

Only the inherent filtration of the tubes was employed, being equivalent to 2 mm Al. On the Practix the normal shielding of a detachable steel cone was used. On the Super-Practix a lead pipe was employed to collimate the beam and minimize stray radiation. The pipe, of 75 mm internal diameter, reached from the x-ray tube to the cephalostat.

Preparatory experiments with films in cassettes with intensifying screens to reduce exposure time produced pictures with a severe deterioration in sharpness due to the screens. Very fine grain industrial films were also tried at the other extreme, but exposure time proved too long in relation to the period of anaesthesia obtainable by simple means. The best results were obtained with Kodak Occlusal Ultra-speed Dental x-ray Film DF45, which therefore was used exclusively in the study described here. The films were identified by writing with heavy pressure on the tube side of the film pack. The pressure leads to transference of the inscription to the photograph upon exposure of the film, and ensures that a permanent record is obtained without difficulty.

Development of the films was for 4 minutes at 20 °C in Kodak Dental x-ray Developer using intermittent nitrogen agitation.

The maximum radiation received by any rat, including those used for additional exposures for determining the method error, was about 5000 mr over a period of 500 days. This is the same order of dosage permitted for human workers. The actual dose rate was determined with a Philips Dosimeter type 37471 and ionisation chamber type 37482/10, and was found to be approximately 15 r/min in the centre of the cephalostat.

The procedure for making the x-ray pictures involved administration of a general anaesthetic except on the day of birth. Ether was used, despite its tendency to induce movements of convulsive respiration. The animal was placed in a glass-covered box in the bottom of which was cotton wool saturated in ether. This method is relatively safe, simple to administer, and has a duration adequate for the procedure. Recovery time is short and uneventful. Despite the relative safety of ether, practically all premature deaths in the experiments occurred during anaesthesia.

Working with three operators it was possible to take 120–140 x-rays in 4 hours. Almost always it was the same person who undertook the same part of the procedure, so that in particular the variation in positioning the rats in the holder was minimized.

The Application of Roentgen Cephalometry

The use of cephalometric x-ray pictures has several advantages over other methods of growth assessment. It presents an accurate record of stages of development in the calcified tissues. Moreover, the internal structure of the head can be studied in detail. With x-rays it is possible to obtain data with a precision that is lacking in other methods used for the study of head growth in living beings. Consequently by using a series of pictures it is possible to follow accurately the changes that occur with time.

Investigations in which the subjects are followed up over a period of time are called longitudinal studies; for that purpose, every individual has to be measured on at least two separate occasions. Besides the general data on the morphology of the subjects at certain developmental stages, longitudinal studies provide specific information on the individual changes that have taken place during the time when records were collected. By appropriate handling of the data of the individual changes, detailed information is gained not only on the average changes but on the individual behaviour as well. In a study of growth particularly, the latter aspect is of great importance.

Longitudinal studies take time and require special attention to maintain as many specimens in the group as possible. However, the extra information obtained warrants this.

Cross-sectional studies, in which every subject is recorded only once, are less expensive and time-consuming. A large amount of data can be collected within a relatively short time. However, to gain an amount of overall information comparable to that obtained in longitudinal studies the group has to be 20 times as large (Tanner 1962). Besides this, cross-sectional studies by their nature cannot furnish any information on individual behaviour.

Frequently studies designed as longitudinal ones do not reach that goal completely. Some subjects drop out, others may join later on, or some of the records collected cannot be used. A study of this nature is called mixed-longitudinal. By proper treatment of the data, the information distilled can arrive quite close to the level of that from a purely longitudinal study.

When differences between two samples are examined, it can be shown that the variance of the difference is equal to the sum of the variances of the samples, minus twice the product of the standard deviations of the samples and the coefficient of correlation between the samples. If, as commonly is the case, the differences between the samples are calculated from random pairs, the correlation is in the long run equal to zero and the last term has no effect. But where the differences are taken between individuals in the sample who exhibit positive correlations, then the size of the term increases and the standard deviation and the variance of the difference may be very much reduced.

This is what occurs in longitudinal studies, when differences are examined between the measurements of the same individual taken on different occasions. The correlation coefficient in these cases theoretically can approach 1, and the variance is consequently much reduced. Any statistic such as the Student 't' test which is based on the properties of the variance will therefore exhibit more efficiency with longitudinal information (Yates 1949). As has been mentioned, this reduction in variance can also be found in mixed longitudinal studies, though to a lesser degree (Tanner 1951, Yates 1949, Patterson 1950). This can be accomplished by employing the characteristics of the longitudinal data to interpolate the missing elements of the incomplete series. This technique was adopted with our material when it was found necessary to discard some of the x-ray pictures for reasons later to be discussed.

Obtaining Data from the x-ray Pictures

The method used to procure measurements from the x-ray pictures was to locate the landmarks required on the film and to record their co-ordinates in a Cartesian system. Location of the landmarks was purely visual. No marks were employed, but the film was traversed beneath the cross-wires of a fixed optical system till the image of the required anatomical structure was at their intersection. By using no marks a form of spurious correlation can be avoided (Björk and Solow 1962).

The equipment (Plate V) comprised the crosstable of a Leitz Durimet Knoop Hardness tester, which was modified to permit traversing double the normal distance on one axis, so as to accomodate the dimensions of an adult rat head. On that axis was fitted a new micrometer calibrated in 0.5 mm $\times 10^{-2}$ with a range of 50 mm. The other axis was fitted with the standard micrometer calibrated in mm $\times 10^{-2}$ with a range of 25 mm.

On the cross-table was fixed a transilluminating base from a Wild dissecting microscope. The binocular body of the dissecting microscope was mounted on a separate rigid stand and positioned over the transilluminating base. To avoid parallax, the body was so tilted that the central ray of the left optical system was perpendicular to the plane of the film held under a glass slip on the transillumination base. The cross-wires were in the left ocular. Periodic checks were made of the adjustments.

The magnification used most of the time was 12 X. This was the best compromise between the decrease in contrast that accompanies increase in magnification, and the greater ease of actual measurement that comes with higher magnification. Variable brightness in the illumination of the films was essential to obtain optimum viewing conditions.

Readings from the micrometers were recorded on sheets laid out to facilitate transfer to punch cards, and were taken to the nearest mm $\times 10^{-2}$. All measuring was done twice without moving the film between one series of measurements and the next. This made it possible to check immediately for reading errors. Originally the films were moved between successive series of measurements so as to remove bias, but as error in reading the micrometers was found to be the most common one of importance, the above procedure was adopted instead.

Error of the Method in Cephalometry

Tests were carried out to see to what extent the method gave rise to variation in the results, and to reduce this as far as practicable.

When observations are made from cephalometric x-rays, the sources of uncertainty can be subdivided (Savara et al 1966). If an individual measurement is taken from an x-ray picture, it will represent the true value of the character, with effects added or subtracted due to the inherent geometric distortion, due to improper positioning of the subject relative to the film, to the degree of precision in locating the points to be measured, and to errors in measuring positions of those points once located. In a series of measurements, additional variation can exist if different operators perform the same task, and there is extra variation when the task is repeated on widely separated occasions.

When a series of measurements is taken, the variation is the combination of the biological variation and the observational variation. For the greatest effectiveness of the measurements, the latter variation should be as small as possible. In circumstances where the biological variation is eliminated as in double determinations, it is possible to show up the observational variation and if desired, partition it into its various components. By this means it is possible to see where the variation originates, with an eye to its reduction.

Reduction of the errors in the cephalometric x-ray system is a compromise between the desired performance and the characteristics of the material. As a rule, the best possible picture is sought by the use of the greatest convenient focus-film distance, the shortest object-film distance, and the smallest target area that will provide adequate output for the shortest possible exposure. In this way the geometric distortion and penumbration is reduced.

Besides this, the reliability of the measurements of the film depend on its quality, necessitating fine grain, good density, proper exposure constants, and correct development. Reduction of secondary radiation gives considerable benefit to film quality. Firm fixation of the subject prevents movement blurring the film, and contributes to a further important factor, positioning of the subject in a constant attitude so that the reference planes are constant in their relation to the films.

Reduction in the variation due to human factors in the use of the equipment is obtained by quality control of the results, by initial standardizing of methods, and by retaining the same experienced operator for each function.

Once the film has been produced, the reliability of the observations is dependent on the variation in locating the landmarks, and in making the actual measurements.

The method was tested by taking exposures of the same 16 rats from two experimental groups on two occasions. Duplicate films were available in the film packs. 30 Points were selected and their co-ordinates recorded for each film. From these data all 435 distances between these points were computed for each film, and the results were compared between duplicates and between films taken of the same rat on separate occasions.

Table I CHARACTERISTICS OF MEASUREMENTS IN MILLIMETRES. ODD-NUMBERED RATS AT DAY 165 EXP. 2

Distance	Group Mean	Standard Deviation	Absolute Location Error	Absolute Overall Error	Relative (%) Location Error	Relative (%) Overall Error	Mean Difference between Duplicates
Nasion-rhinion	17.228	0.516	0.25	0.29	1.45	1.69	0.115
Basion-supradentale	43.629	0.781	0.17	0.16	0.39	0.36	0.006
Length of sphenoid	8.111	0.207	0.08	0.09	1.02	1.08	0.041
Crista limitans-inion	19.780	0.455	0.16	0.18	0.79	0.91	0.014
Parietofrontal suture to spheno-presphenoidal synchondrosis	9.051	0.280	0.07	0.08	0.76	0.88	0.070

Table I* gives an indication of the type of result obtained. From examination of these results it was possible to discard points exhibiting relatively high errors. 15 Points were eventually chosen as the most reliable, to be used in subsequent measurements. Where anatomical significance was great, a point could be accepted that might have a less satisfactory variability.

It became apparent that the positioning error was negligible, and it was felt that improving the location of the points was the only correction needed. All measurements were duplicated in the future work principally for this reason, and the definitions of the points were made as stringent as could sensibly be used.

The points chosen are shown in Plate IV and are accompanied by their definitions.

Processing the Information

Having obtained the co-ordinates of the points, the data were processed in an I.B.M. 360/50 computer in the computer centre of the University of Nymegen so as to provide information on all linear dimensions, some angles, some areas, and the radius of the hypothetical circle describing the maxillary incisor. From this basic information, incremental data were derived; and from fitted curves on the time-distance data, estimates of velocity and acceleration were made. These data were then tested, with the additional data on weight, cranial capacity, adrenal weight, body and tail lengths, and molar sizes, to determine if the experiment had produced any differences between the two groups. The normality of distribution of the data was checked and found satisfactory, using the procedure of graphing some of the data on normal distribution paper. This makes the use of statistics based on normal distribution acceptable.

Correlations were sought between various characters and examined for significant differences between the groups by applying the 't' test to 'z' transformations of the correlation coefficients.

The results of the tests were accepted as significant when the probability of the result being due to chance was less than 5%. Generally, the level of probability was stated, as, though a 5% level is adequate, the very much more decisive levels obtained in many quantities offer better support for any hypothesis.

In this chapter there has been a description of the animals used in these experiments, their treatment, and the way in which their reaction to the experimental conditions was assessed. A brief idea has been given of the type of records accumulated, and of the importance of the use of longitudinal material. The Roentgen cephalometric method has been described in more detail, and information on the error of the method has been given. In conclusion the manner in which the results were analysed has been described.

* Roman numerals indicate tables in the text, Arabic numerals refer to tables in the appendix.

CHAPTER V

CONTINUOUS IMPOSITION OF LITTER-SIZE EXTREMES: EXPERIMENT 2

This chapter will be concerned with the work done on the first series of rats used in these experiments. The name Experiment 2 is used as a means of identification.

The term *Series* applies to both groups of experimental animals together and generally covers the total time-span of the experiments as well. The term *Stage* is applied to the occasion on which a set of observations was made. In the entire collection of experiments the stages correspond fairly well in time across the series.

For convenience, the narrative will be divided into several parts, i.e.

a. Superficial observations
b. Weight
c. Tail/body ratio
d. Cranial capacity
e. Adrenal gland weight
f. Histology
g. Molar size
h. Mandible
i. Skull

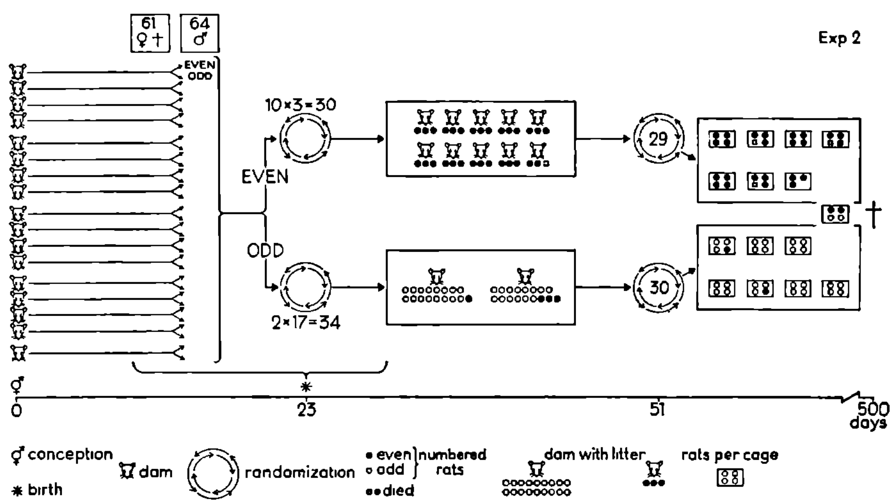


Fig. 1. Schematic illustration of the design of Experiment 2.

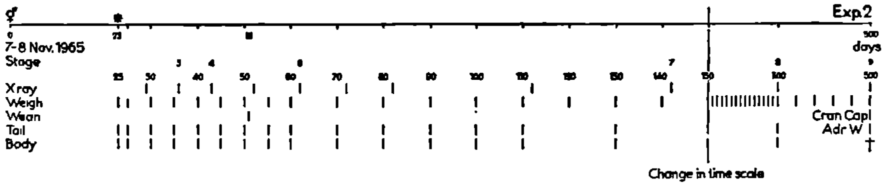


Fig. 2. Experiment 2. Timing of stages and records. Note that x-ray records taken on some occasions were not used, those used being restricted to those taken at the times denoted by a stage number. Cran Cap indicates determining cranial capacity. Adr W indicates determining adrenal gland weight.

GENERAL INFORMATION

The rats of this series were conceived on the night of 7/8th November 1965, and born 30th November i.e. on the 23rd day after conception taking 7th November as day 0. They were immediately redistributed as described in Chapter IV and illustrated in fig. 1. and were arranged in two groups, one composed of 10 reconstituted litters of three rats each, and the other of two litters of 17 rats each. This grouping was kept till day 51 post-conception (day 28 postnatal) when the rats were weaned.

Upon weaning, the rats were placed four to a cage in random distribution, except that the rats of both groups were kept apart. This was done as it was felt that the rats of one group might influence the others. However because the group totals were not divisible by four, two rats of each group did finish up with two of the other, and this did not present problems.

The rats were sacrificed at the post-conception age of 500 days, by overdose of Nembutal followed by formalin perfusion. The heads were removed and skinned, the cranial contents removed by aspiration, and the adrenal glands were dissected out. These items were preserved in 4% formalin in jars identifying each rat.

Mortality was quite light as can be seen from the sample numbers in table 1 (Appendix) and fig. 1. The death rate was only slightly greater in the large litters. Most deaths occured in the first week after birth.

a. SUPERFICIAL OBSERVATIONS

There was no difference seen in the timing of developmental criteria between the two groups. Note was taken of hair formation, opening of eyes, formation of external ears. What was obvious was that the rats in large litters were smaller than those in small litters, and that the former were dirty and unkempt compared with the others. The mothers with large litters seemed more nervous than those in the other group.

The findings of Widdowson and McCance (1960) that the development of the smaller rats was slightly retarded is not confirmed by our results. However our observations were not so frequent nor of as large a number of animals as theirs.

b. WEIGHT

Weight is the most commonly used measure of growth. It is however readily influenced by circumstances not connected with growth, such as diet and exercise, and it changes even when all normal growth processes are finished. Further, it must be remembered that weight is related to the cube of linear dimensions.

In this work, the weight records were taken to determine in a simple way if the experiment caused differences in the animals. The data were also used to investigate the correlation between weight and other dimensions and to compare our results with findings by other investigators.

Method

The timing of weighing was different between this and later experiments. The animals were weighed on day 23, the day of birth. Thereafter they were weighed on day 25 and every 5 days till day 60. Then the interval was made 10 days till day 300. The next weighing after day 300 was performed every 40 days till day 500. See figure 2.

Findings

The mean weights are given for each group in table 1 with the appropriate standard deviations and sample numbers. Student's 't'-tests were carried out to determine the probability that the samples might be from the same universe. The results also appear in the table.

Figures 8 and 10 give a graphic indication of the course of the gain in weight of the two groups. Since it is of interest to compare these with those of the later series, the latter are also drawn on the same figures but will be discussed later. Note that the time scale is transformed to logarithms, for reasons of convenience.

Analysis

The results of the tests of the material indicate that there was a significant difference in weight between the groups, that was apparent two days after the imposition of the experimental conditions and continued through the whole period studied.

It is interesting to note the close correspondence in general pattern of the curves of both groups.

Discussion

Comparison of these results with those published by Jackson and Stewart (1920), Widdowson and McCance (1960), Dickerson and Widdowson (1960), Widdowson and Kennedy (1962), Widdowson and McCance (1963) shows general agreement as far as can be seen from their rather summary data. Close agreement is hardly to be expected since only Jackson and Stewart used Wistar rats, and then many years ago, and their experimental arrangements differed from ours. Nevertheless it can be seen that in our rats divergence from the course of growth was produced in the rats of the large litters, by which their weight became about 60% of that of the other group at day 40. The proportion rose to about 80% by day 100 and remained in the region of 85% till 500 days, when the experiment ended.

The data on weight of Wistar rats due to Freudenberger (1932) are taken as a modern norm (Biology Data Book 1964). They indicate that our small animals correspond fairly well with his animals, whereas our large animals exhibit weights considerably in excess of these.

Conclusions

The experimental conditions gave rise to a difference in the weights of the subjects, the animals in large litters being permanently reduced in this measurement when compared with those from small litters. As rats from large litters do not seem to differ from average Wistar rats of 1932 with respect to growth in weight, one may conclude that their environment was not excessively severe.

There was a definite degree of recovery towards the other group, by the smaller rats, which was most noticeable up till about day 150 post-conception.

C. TAIL/BODY LENGTH RATIO

The ratio of tail length to body length is of interest in that it gives in a relatively simple way, the possibility to determine a change in body proportions.

Method

The measurements were done at the same time as weighing, until day 110. See figure 2. The animals were by then difficult to handle so the interval was increased to 20 days for the next two records. Then no further measurement was done till day 300, when they were again measured while recovering from the anaesthetics given for the cephalometric x-rays taken at that time. No further measurements were taken.

The instrument used is illustrated in fig. 3. Different sizes of slots were used (3, 5, and 10 mm) to accommodate increase in tail thickness. The rat's tail was placed in the slot and was drawn through till the haunches were against the plate. The distance to the tail tip was read off the scale in millimeters. After flattening the body of the rat against the other scale, the distance was taken to the tip of the nose.

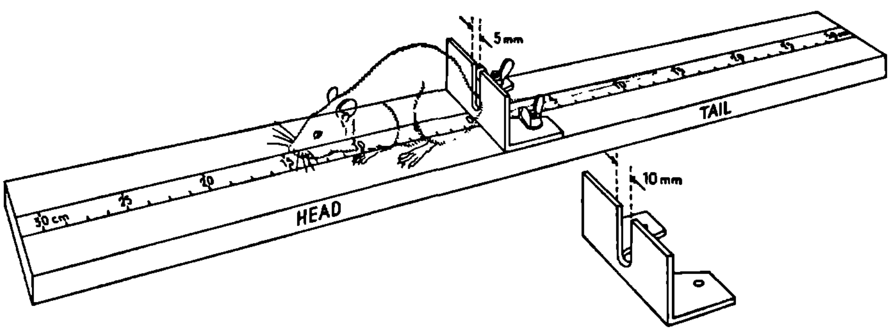


Fig. 3. Tail and body length measuring instrument with interchangeable tail-slot plates. A slot of 3 mm was also used but is not shown.

Findings

Tables 5, 6, and 7 and figure 9 illustrate the findings.

Analysis

In the case of the rats from large litters the tail becomes a greater proportion of the body length than in rats from smaller litters. Since the tail length shows little final difference between the groups, the implication is that growth of the tail is less affected than that of body length. This is apparent from the records on day 25 and continues throughout the experiment.

After 2 days of the experimental intervention, there was a significant difference in the body lengths that continued to exist. Regarding tail length, the same phenomenon was observed from day 30 till day 150.

In both groups the ratio between body and tail lengths showed a change with age. (Table 7). As the rats grew older the tail became almost as long as the body, instead of being (as at birth) only about 40 % of that length. Compare the rats of the next experiment in plate II at different ages.

A significant difference in tail/body ratio was present between both groups at all registration dates except on day 80.

Discussion

The only applicable longitudinal study of tail and body length in rats to have been found is that of Acheson et al (1959). Their rats were of Sprague-Dawley strain, and showed a relatively longer tail than Wistar rats. Freudenberger (1932) gave normal tail/body ratios of male Wistar rats as .34 in newborn, .83 21 days after birth, (day 44) .99 after 144 days, (day 167) and .95 at one year after birth. This was from cross-sectional data of at least 30 rats per age group but the technique used was not specified.

Dickerson and Widdowson (1960) give a graph of tail length over total length, but this is based on cross-sectional material in very small groups and measured after death. It is assumed they measured nose to anus as described by Widdowson and McCance (1960). They found no significant difference in ratio between rats from large and small litters. This readily can be attributed to the paucity of the information available to them, since their basic data do not conflict with ours. They were probably better justified if they had said no difference could be detected, rather than state as they did that the ratio of tail and body length was not altered by accelerating growth.

Summer (1915) had noticed that smaller mice had proportionally longer tails than did larger ones. Jackson and Stewart (1920) made the same observation on rats though they confused the issue a little by comparing animals of the same weights rather than ages, and by mixing sexes. Their data also suggest a catch-up in tail/body proportions on refeeding animals that had been underfed from birth.

Jackson (1937) gives tail and body lengths after refeeding rats kept on a maintenance diet from weaning. The ratio was .918 for large (control) rats and .927 for small rats after about day 450.

The inter-group difference in final body length contributed by the head in our work is probably only about 2 mm, as will be seen from later results. So one can assume that the remainder of the difference of about 8 mm does express a difference in reaction of the tail and the body of the rat to the same environ mental change.

Conclusion

The ratio tail/body length is significantly lower in rats from small litters, and the inter-group difference in tail lengths is less than in body lengths. From this can be concluded that the influence of the experimental conditions was greater on the body than on the tail.

d. CRANIAL CAPACITY

To check the correspondence between actual measurements of cranial capacity and the longitudinal measurements taken to represent this characteristic, measurements were made after aspirating the cranial contents but leaving the dura intact. This was done by weighing the amount of mercury required to fill the cavity.

Method

Aspiration was done directly after removing and skinning the head. The fixed heads were drained for a few minutes and mercury was poured into the foramen magnum till a standard meniscus was created. The mercury was then weighed to the nearest milligram on an E. Mettler balance model H. 16 (80 gm capacity in gm $\times$ 10^{-5}.)

This was repeated four times, and the mean was used in calculations. From these determinations, the standard error of the difference in replicate measurements was found to be 0.091 gm.

Findings

Table 8 gives the means and standard deviations of the measurements, and the correlation between body weight and cranial capacity, treating the experimental groups separately. The results are tested by Student's t-test; in the case of the correlations, after applying Fisher's z-transformation.

Analysis

There is a significant difference in the cranial capacities between the experimental groups. There is also a positive correlation between body-weight and cranial capacity in large litters. The correlation between body-weight and cranial capacity of individuals exhibits no significant difference between groups.

Discussion

Smith (1935) gave cranial capacities from measuring the brain directly, that agree quite well with our results. Jackson and Stewart (1920) showed a smaller brain weight in rats underfed from birth, as compared with normals. They found a less significant difference in rats underfed after weaning. Diamond et al (1965) showed that the correlation between body-weight and cranial capacity lost significance with age, and we will return to this when discussing a later experiment from our work. McMeekan (1940) found a relative reduction in brain-weight in his work on pigs on a low plane of nutrition from birth onwards.

This is all evidence to indicate that the growth of the brain is indeed susceptible to the influence of external conditions, regardless of its important functions.

The effect of the experimental difference on our animals, measured at quite an advanced age for a rat, revealed that a permanent difference had been induced in the cranial cavity by the conditions prevailing before weaning. The relationship between cranial capacity and weight might be seen as rather close in the large-litter animals.

Conclusions

The experimental differences gave rise to differences in cranial capacity in our rats, so that the smaller rats had the smaller values. There was a significant positive relationship between body-weight and cranial capacity in the large litter animals.

e. ADRENAL GLAND WEIGHT

An indication of any difference in the size of the adrenal glands was sought as such a connection between the environment of the animal and the activity of these glands is interesting.

Method

The adrenals of individual rats were carefully stripped of their fat and were placed on filter-paper to absorb excess moisture. They were then weighed four times on the Mettler H. 16 balance, to the nearest 0.1 mg.

There was no difference detectable between replicate weighings.

Findings

Table 8 gives the weights of the adrenal glands of both groups as means and standard deviations. The correlation between body-weight and adrenal weight is given. The weight is also expressed as mg/100 gm body-weight.

Analysis

There is a significant difference at day 500 between the weights of the adrenal glands of the experimental groups. The large-litter rats have smaller adrenals. No significant correlation between the body-weight and adrenal gland weight within the groups is found. The adrenal gland weight expressed as mg/100 gm body weight shows no difference between the groups.

Discussion

Stress is a cause of adrenal gland enlargement, and starvation is a form of stress. It is not unexpected then that a relative enlargement is found in the adrenal glands of animals subjected to starvation (Johannessen 1965, Jackson 1919). This reaction would of course be in the opposite direction to other growth trends in underfed animals.

The reaction in the animals examined does not show this relative change, but this can be accounted for by the long interval between the imposition of the stress and the examination of these organs. It is indeed interesting to see that the proportion of the adrenals to body weight was so similar in both groups, as far as the mean values are concerned. The period elapsed between the imposition of the effect and the examination of the result may have concealed any reaction. If there had been a reaction, the recovery seems to have been complete.

Conclusions

There is a significant difference in adrenal gland weights, the large-litter rats having the smaller glands at day 500.

The experimental conditions resulted in no detectable difference in the mean proportions of adrenal/body weight between the groups.

f. HISTOLOGY

It was unlikely that systematic histological differences could be found between the groups (Paynter and Grainger 1956). Nevertheless it was felt meaningful to include a histological evaluation of some rats in this study to check if this assumption were true.

Method

The skulls of 3 rats of each group, and adrenal glands of 6 animals of each group, were sectioned. The animals had been fixed with formalin perfusion at sacrifice, and were kept in 4% formalin solution. The heads were divided sagitally with a fine saw-cut at a level just lateral to the maxillary molars, were decalcified, embedded in paraffin and sectioned at 15 microns. The plane of section was as nearly as possible parallel to the midsagittal plane. The skulls were stained with H and E, and tri-chrome (Goldner). The adrenal glands were stained with H and E or left unstained after sectioning on a freeze-microtome and mounted without the use of fat solvents. This was to permit examination for lipoids, by polarized light.

Findings

The sections showed no difference between the groups regarding any of the aspects studied.

The features were those that would be expected in any rats in normal conditions at the age of 500 days. The rat no. 9 which had a defective generating organ of the upper incisors, seemed less mature than the normal rats. Particularly, there was no trace of calcification of the basilar synchondroses, and the cartilage cells therein, although in disorder, were perhaps more in columnar arrangement than those in the other animals studied.

Conclusions

No systematic histological differences could be found that could be attributed to the experiment.

The conditions found in rat no. 9 were not attributable to the experiment, and are of interest as an incidental glimpse at the possible effect of prolonged feeding difficulties. Its tissues gave an impression of being less mature than those of the other experimental rats.

g. MOLAR SIZE

The teeth can be considered as permanent records of the history of development of an individual. So the study of the rat molars and incisors was of particular interest regarding the question of what effect the experiment might produce in that field.

Method

Lateral x-rays taken on day 82, were used for this purpose. Of each group 28 were taken and the mesiodistal diameters of the maxillary molars were measured by traversing the films on the cross-table of the Durimet microscope, with one axis of the table parallel to a line joining a mesial measuring point on the first molar with a distal measuring point on the third molar. The mesial measuring point was taken at the greatest contour near the cervical margin of the enamel, and on the third molar the distal measuring point was located at the most distal point on the distal contour, generally being near the marginal ridge.

Double determinations were carried out to assess the standard deviation of the error of the method according to the formula $s_1 = \sqrt{\frac{\Sigma d^2}{2n}}$ (Dahlberg 1940). It was found to be in the order of $\pm$ 0.05 mm.

Findings

The findings are reported in table 9. The results of Student's t-test are given.

Analysis

There is a significant difference in the mesio-distal length of the molars of the two groups of rats, taking the total molar segment and also the individual lengths of the second and third molars.

Discussion

According to Schour and Massler (1949), the calcification of the rat molars begins with apposition of dentine 21 days after conception, in the first mandibular molar. The second molar follows at day 23, the third at day 35. Maxillary molars follow one day later than their counterpart in the mandible, according to those authors. Interpretation is difficult since they do not state the post-conception date of birth; and the days given here assume that their rats were born on day 21.

Calcification of the crowns can be seen on x-rays taken on day 25 (M_1), day 30 (M_2), and day 43 (M_3). Completion of crown formation is given as days 33, 34 and 43 respectively. Johannesen (1961) gives a time 1 day earlier for his rats' mandibular 1st molars. Paynter and Hunt (1964) indicated how the growth of the dental papilla of the maxillary first molar was very rapid between the 18th and 23rd days, slowing at the time the dentine first appeared in the cusp tips. They described how the mesio-distal width increased slightly till day 31, and attributed this to the growth in crown length along the diverging mesial and distal surfaces. It is likely that one can assume that the growth in mesiodistal dimension of the other molars is in the same way related in velocity to the time of commencement of dentinogenesis. It should be noted that Paynter and Hunt found that dentinogenesis began between days 22 and 23, for M_1 max. It can also be seen that their rat molars grew in mesio-distal dimension from 2.06 mm to 2.62 mm in the interval from day 23 to day 31, when measured at the dentino-enamel junction. It would then be quite possible for an alteration in growth rate in our experimental groups to express itself in a difference in crown size in rat molars even when the intervention occurred after birth, with a chance of a marked effect in the second and third molars which could be affected while the papilla was in a period of rapid growth. The difference found in our investigation may be interpreted in this way.

Comparable morphological effects due to nutrition have been noted by others (Paynter and Grainger 1956, Kruger 1962, Tonge and McCance 1965). A tendency can also be found in the data of Outhouse and Mendel (1933) where the total molar length in optimally fed rats exceeds that in 'normal' rats. The present work provides adequate data from animals not suffering from a detectable deficiency in the quality of their diet.

Conclusions

The experimental conditions brought about a difference in the mesio-distal length of the maxillary molars of the two groups of rats, statistically significant in the case of the overall length and the individual lengths of the 2nd and 3rd molars.

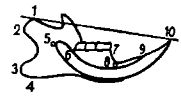
h. MANDIBLE

The cephalometric examination of the mandible was undertaken on the x-rays obtained at two stages. In that way the growth of the mandible could be studied and related later on to that of the rest of the skull. More frequent examination was not felt to be practicable because of the extreme difficulty of locating useful landmarks in the mandible particularly on younger rats.

The material used was pure longitudinal – each rat used provided data from both stages.

Method

Cephalometric lateral x-rays taken on post-conception days 62 and 142 re-



present stages 6 and 7 (see plate III). These films gave reasonably clear pictures of the mandible during a period of active growth. Films were chosen from each group at each stage for good quality of image and so pairs of films of 19 odd-numbered rats and 15 even ones were obtained.

As shown in plate IV 10 points were located on the films and from their co-ordinates the following quantities were computed for each rat at each stage:
Distances between all points
Vertical distances from points 3 and 4 to line 1 10, and from point 4 to line 6 7
Areas enclosed by points 1 3 10, 5 8 3, 1 4 9, 1 4 10
Angles 4 10 1, 3 5 8, 5 8 3, 4 7 2, 4 7 8, 4 7 9, 7 8 9, 5 9 7, 4 9 7, 8 9 2, 6 3 1, 6 3 8, 3 2/10 1, 6 7/1 10, 4 9/1 10, 8 5/10 1.

Findings

Table II gives the sample numbers, mean values, standard deviations, for the odd and even-numbered groups at both stages, with the increments and relative increments between the stages treated in the same way. The results of Student's t-test of the differences between the two groups and the corresponding probability values are given.

Only a selection of the findings is included, chosen according to the anatomical significance of the quantities, rather than according to any behaviour of the dimensions.

From the mean values, outlines of the mandibles were constructed by triangulation. These are reproduced in fig. 4.

Analysis

Examination of the superimposed outlines provides a good indication of the changes found between groups and between stages.

In stage 6 (day 62, 39 days after birth) all linear dimensions with the exceptions of 3 4 and 5 6 were significantly larger in the small-litter group.

Angular measurements showed no significant difference between groups, except angles 1 10 4, 3 5 8 and 5 9 7.

The areas measured were significantly larger in the small-litter group.

In stage 7 (day 142, 119 days after birth) the degree of difference present between the groups corresponded with that in stage 6 in such a way that the t-value was about 2.0 standard errors less.

Dimensions now showing no significant difference between groups were 2 6, 2 8, 8 9, 7 10, 8 10, 9 10 and 4 to line 6 7.

No angle showed a significant difference between the groups at stage 7.

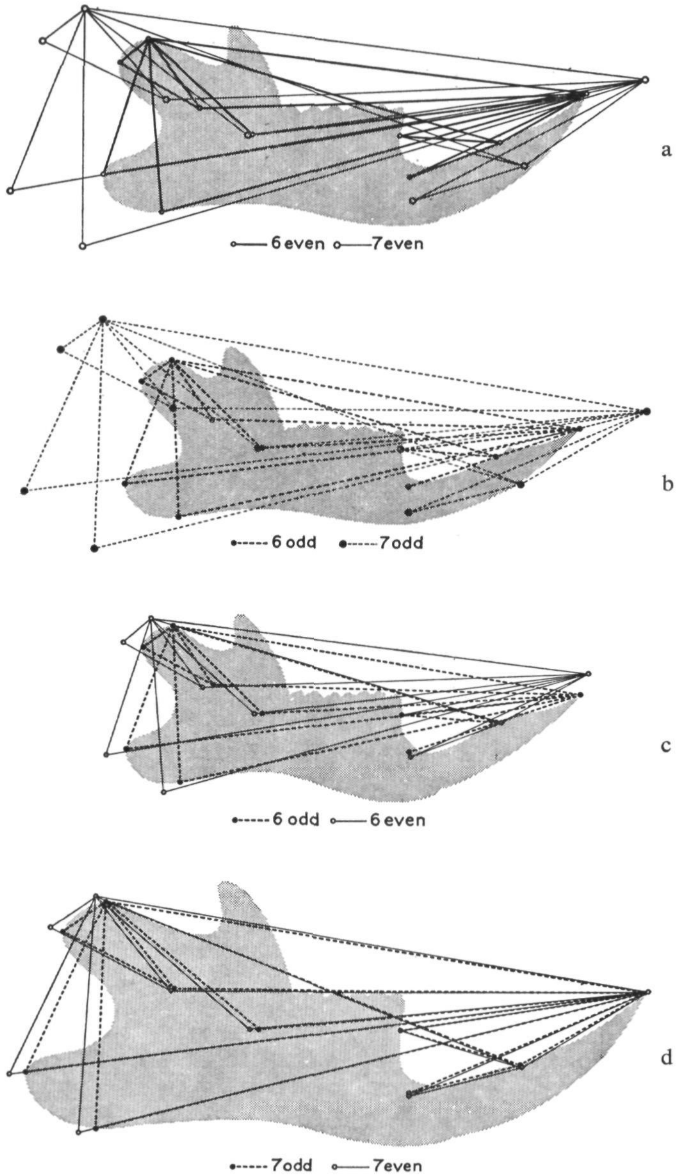


Fig. 4. Experiment 2. Superimpositions of outlines of the mandible, constructed from mean values, oriented on line 6 7, registered on point 7. Construction lines have been left since they help convey the manner in which the changes occur.

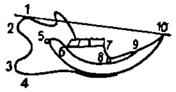
a. Even (small-litter) stage 6 (day 62) on stage 7 (day 142).
b. Odd (large-litter) stage 6 (day 62) on stage 7 (day 142).
c. Odd (large-litter) on even (small-litter) from stage 6.
d. Odd (large-litter) on even (small-litter) from stage 7.

Table II MANDIBULAR MEAN QUANTITIES Sample number 15 even 19 odd in all cases $p<0.05 = *$ $p<0\ 01 = **$ $p<0\ 001 = ***$

Quantity	Small-litter rats Mean Value mm	Small-litter rats Standard Deviation	Large-litter rats Mean Value mm	Large-litter rats Standard Deviation	't'	p
Perpendi-	4 10	0 316	3 62	0 238	4 9	***
cular	5 52	0 359	5 25	0 487	1 7	
4 to 6 7	1 42	0 111	1 63	0 101	—5 8	***
	0 35	0 128	0 45	0 127	—2 3	*
Perpendi-	8 74	0 483	7 94	0 441	5 0	***
cular	12 16	0 343	11 67	0 345	4 2	***
4 to 1 10	3 42	0 093	3 73	0 119	—8 3	***
	0 39	0 058	0 47	0 087	—3 1	**
	degrees		degrees			
Angle	22 81	0 947	22 21	0 577	2 3	*
1 10 4	23 91	0 821	23 71	0 476	0 9	
	1 10	0 212	1 50	0 173	—6 1	***
Angle	160 88	1 139	160 86	0 715	0 1	
4 9/1 10	161 76	1 028	161 75	0 630	0 0	
	0 88	0 223	0 89	0 197	—0 1	
Angle	172 88	0 910	172 30	1 333	1 4	
6 7/1 10	170 88	1 516	170 78	1 853	0 2	
	—2 00	0 471	—1 52	0 465	—3 0	**
Angle	89 56	3 208	90 23	3 472	0 6	
3 2/1 10	85 21	1 874	85 62	4 158	0 4	
	—4 34	0 972	—4 61	1 401	0 6	
	mm^2		mm^2			
Area	77 91	4 890	66 12	5 643	6 4	***
1 9 4	137 12	5 930	126 95	4 900	5 5	***
	59 21	1 089	60 82	1 535	—3 4	**
	0 76	0 078	0 92	0 167	—3 4	**
Area	27 69	1 365	24 63	1 930	5 2	***
3 5 8	49 33	3 078	46 67	2 324	2 9	**
	21 64	0 600	22 04	0 571	—2 0	
	0 78	0 079	0 89	0 148	—2 6	*

Quantity	Small-litter rats Mean Value mm	Small-litter rats Standard Deviation	Large-litter rats Mean Value mm	Large-litter rats Standard Deviation	't'	p
1 4	8 94	0 439	8 13	0 420	5 5	***
	12 24	0 361	11 78	0 284	4 2	***
	3 30	0 091	3 65	0 106	—10 2	**
	0 37	0 052	0 45	0 077	—3 5	**
2 10	23 85	0 478	22 40	0 690	6 9	***
	30 80	0 677	30 02	0 384	4 2	***
	6 95	0 135	7 63	0 153	—13 5	***
	0 29	0 023	0 34	0 039	—4 4	***
3 4	3 42	0 292	3 18	0 382	2 0	
	4 61	0 505	4 60	0 442	0 05	
	1 19	0 127	1 42	0 123	—5 3	***
	0 35	0 154	0 45	0 204	—1 6	
3 8	15 50	0 278	14 36	0 561	7 7	***
	20 36	0 534	19 60	0 468	4 4	***
	4 86	0 133	5 24	0 139	—8 1	***
	0 31	0 035	0 36	0 053	—3 2	**
3 9	20 20	0 310	18 90	0 684	6 9	***
	26 15	0 759	25 27	0 608	3 8	***
	5 95	0 185	6 38	0 171	—7 0	***
	0 29	0 036	0 34	0 048	—3 4	**
3 10	24 96	0 443	23 32	0 790	7 2	***
	32 90	0 775	31 94	0 689	3 8	***
	7 94	0 167	8 63	0 189	—11 1	***
	0 32	0 027	0 37	0 044	—3 9	***
2 9	19 75	0 364	18 51	0 605	7 0	***
	25 24	0 682	24 47	0 303	4 4	***
	5 48	0 149	5 96	0 139	—9 7	***
	0 28	0 030	0 32	0 042	—3 1	**
2 5	4 71	0 308	4 13	0 349	5 0	***
	6 93	0 601	6 48	0 348	2 7	**
	2 22	0 119	2 35	0 108	—3 3	**
	0 47	0 034	0 55	0 151	—1 7	

	Quantity	Small-litter rats Mean Value mm	Small-litter rats Standard Deviation	Large-litter rats Mean Value mm	Large-litter rats Standard Deviation	't'	p
Stage 6	5 8	11 19	0 330	10 58	0 397	4 8	***
Stage 7		13 41	0 258	13 20	0 195	2 7	**
Increment		2 22	0 085	2 62	0 082	—13 9	***
Rel Increm		0 20	0 034	0 25	0 042	—3 8	***
Stage 6	7 8	2 21	0 109	1 97	0 118	6 0	***
Stage 7		3 45	0 168	3 34	0 211	1 6	
Increment		1 24	0 045	1 37	0 052	—7 7	***
Rel Increm		0 56	0 093	0 69	0 136	—3 2	**
Stage 6	8 9	4 93	0 184	4 73	0 240	2 6	*
Stage 7		5 99	0 354	5 88	0 246	1 1	
Increment		1 07	0 082	1 15	0 066	—3 2	**
Rel Increm		0 22	0 066	0 24	0 068	—0 9	
Stage 6	9 10	5 10	0 221	4 66	0 233	5 5	***
Stage 7		7 59	0 250	7 45	0 268	1 6	
Increment		2 50	0 064	2 78	0 069	—12 1	***
Rel Increm		0 49	0 135	0 60	0 084	—2 9	**
Stage 6	7 10	9 78	0 281	9 21	0 358	5 1	***
Stage 7		12 85	0 374	12 69	0 305	1 4	
Increment		3 08	0 072	3 48	0 071	—16 2	***
Rel Increm		0 31	0 031	0 38	0 044	—5 2	***
Stage 6	6 7	7 52	0 150	7 13	0 151	7 5	***
Stage 7		7 73	0 246	7 30	0 302	4 5	***
Increment		0 21	0 078	0 17	0 066	+1 62	
Rel Increm		0 03	0 041	0 03	0 040	−0 3	
Stage 6	5 7	10 24	0 311	9 75	0 310	4 6	***
Stage 7		11 99	0 240	11 79	0 238	2 5	*
Increment		1 75	0 089	2 04	0 082	—9 9	***
Rel Increm		0 17	0 038	0 21	0 042	—2 9	**
Stage 6	5 6	2 95	0 282	2 90	0 228	0 6	
Stage 7		4 54	0 272	4 77	0 212	—2 75	**
Increment		1 59	0 075	1 88	0 074	—11 3	***
Rel Increm		0 54	0 134	0 65	0 153	—2 2	*



The areas still were significantly larger for the small-litter-size rats.

The increments and relative increments reflect this state of affairs. All indicate a catch-up in growth of the large-litter rats, in every dimension; significantly greater increments were found in large-litter animals for most linear dimensions.

The dimension 6 7 shows no increment to speak of, being closely related to total molar length.

Angles show no changes which reveal significance.

In comparing the mandibles of the two groups at stage 7, it is apparent that there is very little difference in the part anterior to the mental foramen either in linear or angular measurements. However, there is considerable difference in the ramus height (1 4), and in the total mandibular length (2 10), which have so remained in proportion that the shape of the mandible stayed more or less the same, as expressed by the enclosing angle 1 10 4.

Where changes in form have occured, as e.g. in the caudad movement of the angular process relative to the condyle (angle 3 2/1 10), these have been in the direction of reducing the difference between the groups.

Discussion

The fact that the mandible does not lie in the midsagittal plane has been realised. Possible distortion of the measurements due to changes relative to the midsagittal plane were considered negligible.

No comparable longitudinal material has been discovered. Much has been written about the possible susceptibility of the condyle to external influences, with manifestations in deformity of the mandible. There has also been work which seems to show a difference in response of endochondral and periosteal bone growth to unfavourable conditions of nutrition (Park and Richter 1953). But in our material no significant difference shows up in form of the mandibles, despite the apparent dependence of the condyle on cartilage and of the rest on periosteum for its growth. This takes particularly into consideration the fact that there is no significant difference between angles 4 9/1 10 of both groups, and that, in stage 7, angles in both groups show no significant differences. Undoubtedly however, the experimental conditions had had an effect on shape earlier on.

Conclusions

Although the experiment produced differences in the size of the mandibles, there was very little difference demonstrable in the form. With the passage of time from day 62 to day 142 many differences in the mandibles were reduced, but a size difference remained particularly in the overall lengths. The segment anterior to the mental foramen showed a more or less full recovery from the size difference. A size difference in the molar segments could be demonstrated and as would be expected, this did not change noticeably from stage 6 till 7.

Absolute as well as relative rates of growth in the rats from large litters were shown to be greater than those from the other experimental group.

i. SKULL

Introduction

In the following description, the mandible is not taken into consideration.

The changes in skull growth, as they pertain to the experimental conditions, are the findings of most particular interest in this work. Besides this some observations will be made on the general pattern. Although the value of measurements made in three dimensions is acknowledged, we limited ourselves to the investigation in the mid-sagittal plane. Nonetheless, the presentation of the information gathered involved practical difficulties. It was felt that only the essentials should be given and then in the most condensed form that would not reduce the digestibility. Out of the ways in which this type of data can be expressed, preference was given to the combination of tables and graphic illustrations. The latter are arranged in such a way that fairly exact information can be obtained from the plots regarding mean values, variances, and the existence of significant differences between the two groups at the different times of registration. The plotting of the standard error of the mean on each side of the mean at each registration of it, indicates a significant ($p < 0.05$) difference between the two groups if the values are separated by more than twice the sum of the standard errors. Besides significant differences the graphs also present a good visual impression of the changes with time, and clearly indicate what the course of growth has been during the experimental period.

Plots are given for the mixed longitudinal means of the two groups, and for six individual rats. The latter were selected to represent more or less the extremes of each group. Rats nos. 9 and 60 were included since they had experienced serious under-feeding due to faulty tooth development. They had lost much weight compared to their fellows, with weights of 158 and 130 grams respectively at 300 days; the average for their groups being 346 and 415 grams. It is interesting to note that they were from each experimental group. Unfortunately no. 60 was eaten by his cage-mates on day 335 but no. 9 was sectioned after sacrifice and the findings are reported in the histological part.

In every part to be discussed, a survey is presented indicating for which dimensions tables are given, for mean values measured, and longitudinal increments, and where they and the appropriate graphs are to be found. The increments are the expression of the changes from one time stage to the next, measured exclusively on animals present at both stages, hence 'longitudinally'. $\Delta x(t_{h, h+1}) = x(t_{h+1}) - x(t_h)$.

It might be noted that for orientation in the illustrations, a stage 1 was included in the incremental calculations in the experiment, using data from Experiment 7 as base-line on the assumption that the rats at birth were comparable in all experiments. This resulted in a cross-sectional basis for the first increment which though of a slightly dubious character gives nonetheless a valuable insight into the changes probably occurring at that early period.

Tables of the measured values are, in general, not given. It was felt that only one mean value for each group in each series for each dimension, the one from stage 6, would suffice. All the increments are given with this, so that means at other stages can be calculated if desired. Information regarding the differences or otherwise between the groups is provided in the same table, first for the increments in the form of t-values for the difference between the mean increments from each stage to the next, and second by t-values for the mean measured dimensions, at each stage. In the case of linear dimensions relative increments are included in the tables, but no t-tests were done $\left(\text{rel. incr.} = \frac{\Delta x(t_{h, h+1})}{x(t_h)}\right)$ Comparison of increments is only easily possible between the same stages, as the periods are not of equal length.

The graphs of means are presented using a logarithmic scale for the time values. On the y-axis an arithmetic scale was used except for a few occasions (figs. 13 to 16) in which a logarithmic scale was applied to illustrate relative increments. It may be remarked that the logarithmic time scale is generally used here only as a convenient means of reducing the length of the drawing, with the units on the y-axis so scaled arithmetically that the graphs have all the same height. Care should be taken to note this scale every time, since it may otherwise give a misleading impression of changes. It has the advantage that the form of the curves is readily comparable. One should also note that the last registration on the graphs of means is from stage 8. This is due to the manner of computing the mixed-longitudinal data, which did not include the cross-sectional data of the last stage. The small changes in the last stages make this omission unimportant, particularly as all stages are included in the numerical information presented. The graphs of the individuals, however, are arranged so that the scales match from this experiment to the next, which is not the case in the mean graphs.

By presenting the information in tables and graphs in such a way that for all essential measurements the details are readily accessible, it was felt that the text could be limited to the main aspects of the subject. This will make the analysis of the data more readable, while the more interested reader still has the possibility to study many aspects in detail and see how the mean trends lie, where significant differences between the groups were found, and how some individual rats behaved. After every analysis of the principal aspects a short introduction will be given to the information present in graphs and tables. The findings and analysis will be treated in groups related to the cranial vault, the cranial base, the elements of the face, and the teeth; and the relation between these parts will be gone into.

Method

In the evaluation of Experiment 2, the lateral cephalometric x-rays were used which were taken on days 36, 43, 62, 142, 300, and 500, post-conception. Films taken on day 23 for a later experiment (Experiment 7, chapter VII) were also used, for stage 1, as mentioned above. As previously mentioned, and illustrated in plate IV and at the top of the relevant pages, 15 points were located and their co-ordinates obtained for computation. Note that these points have no connection whatever with the mandibular points though similarly numbered. The following quantities were calculated:

All distances between the points, of which graphs of 33 are published.
Areas bounded by lines joining points:
2 3 4 5 6 7 8 9 2, 2 3 9 2, 1 2 9 8 7 10 14 1,
2 9 14 1 2, 7 8 9 14 10 7

Angles formed by the following points, intersecting at the second point given, or where two pairs of points are given at the intersection of the lines through each pair:

1 2 5	6 3 1	7 8/10 14	5 6 1	4 7 2	
*1 2 6	6 3 8	7 8/ 1 9	5 6 3	4 7 8	
*1 2 8	6 3 11	7 8 /1 2	5 6 4	4 7 9	* = not repro-
*1 2 9	*6 3 14	7 8 /6 1	5 4 3	4 7/6 1	duced in
1 2 10	6 3/4 7	7 8 /6 2	5 9 7	4 7/3 9	graphs here
1 2 11	6 3/10 14	7 8 /6 5	4 9 7	4 7/3 8	
1 2 14	7 8 9	14 7 11	*8 9 2		

The radius of the circle passing through points 12 14 15
Correlations were calculated between cranial capacity determined as in section D, and the area 2 3 4 5 6 7 8 9 2, at day 500.

From the mean values the computer reconstructed outlines of 'mean rat' skulls for the whole series. These were superimposed as indicated in the pertinent captions (figs. 11 and 12). Location of the graphs is simplified by identification by number-letter coordinates on the pages, this being given in the pertinent surveys of quantities.

CRANIAL VAULT

The cranial vault is the part of the skull that envelopes the brain, without the cranial base. It here is delineated by points 2 to 6 inclusive, though 5–6 is properly occiput.

The size and form of the cranium and its relationship to the cranial base will be discussed in this subsection.

Findings

The findings are presented in the form just described. Table III indicates what pertinent data are given, in what form, and where it is to be found. In fact, as mentioned elsewhere, all computed quantities were studied, but for practical reasons a limit had to be placed in transmitting the information.

Table III CRANIAL VAULT DATA LOCATION **EXPERIMENT 2**

										Angles							Areas	
Quantity	23	25	26	34	37	39	45	47	57	125	543	563	654	78/12	78/62	63/47	2392	23456 7892
Table Longit. Incr.	15	16	17	23	24	25	26	27	28	52	40	58	39	48	50	35	55	54
Graph Means Log-Log			*		*	*		*	*									
Graph Means	E1	E2	E3	G1	G2	H3	I1	I3	J3	N3	U1	T2	T3	R3	S2	Q1	B2	B1
Graph Individuals		a3	b1	b3				c1		e1	f3		f2	j2	i3	h3	g3	g1

Analysis

In analysing the data, the general behaviour and the difference between the experimental groups will be considered.

In general the size of the calvaria develops most in the early weeks of life, and this is particularly noticeable in the height dimensions (see figs. 13 to 16). Furthermore the antero-posterior dimensions keep on increasing, whereas relatively little change occurs in the height dimensions after stage 6 (day 62). The form of the cranial vault is initially bulbous, and with age this is changed progressively to a straighter contour. The angle at inion becomes more acute with age, but after stage 6 returns more or less to its original size. The relationship between the body of the sphenoid bone and the axis opisthion-nasion (6 2) is remarkably constant after stage 3 (day 36). Other constructed axes vary in their relationships so as to indicate an elongation and flattening of the cranium without more than slight angular changes in relationship with the sphenoid bone. Comparison between the two groups regarding size reveals that they grow in the same general pattern, but with a difference in intensity. Almost all linear dimensions are found to be significantly greater in the small-litter rats. This difference is less apparent in the height dimensions, and does not show up at all in the posterior cranial height (4 7).

The mean rat outlines give ample illustration of these aspects (figs. 11 and 12). Regarding the group differences in shape, it is found that large-litter rats have the more bulbous crania, though the difference decreases with time. The caudal end of the cranium has also a less acute angle at inion in those rats. Regarding the increase in areas a comparable general pattern is found in the two groups, that corresponds with the changes in the linear dimensions. From stage 3 on, a significant inter-group difference in both the two recorded areas is found. Inter-group differences are slight in regard to the relationship between the cranial vault and the cranial base and those that occur place the large-litter rats in a category with less elongated heads than the other group.

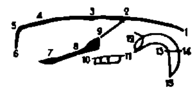
If the study of the tables and graphs is undertaken, particular attention may be paid to size and to velocity of growth, and to the different types of growth to be observed. For this the graphs should be studied individually and in combinations.

In particular, the frontal bone (2 3, graph E1), the parietal bone (3 4, graph G1, b3) and the interparietal bone (4 5, graph I1) may be regarded. The combination of these three, also represented by the total cranial length (2 5 or 2 6, graphs E2 or E3), illustrates how the marked differences in behaviour between the individual parts are more or less compensating for each other so that the end result is a reasonably even growth of the total cranial length over the whole period studied.

Furthermore, it is interesting to look at the values expressing the height of the cranial vault (4 7 and 3 9, graphs I3 and H3).

Then it will be seen clearly that the distance between the interparietal-parietal suture and the spheno-occipital synchondrosis (4 7) behaves almost identically in both groups, showing for example, no noteworthy growth after stage 3, whereas in contrast to this a marked difference shows up between both groups in the more anterior representative of cranial vault height (3 9, graph H3) at stage 4 and onwards. This fits in with the remarks made above regarding the change in shape of the vault and illustrated in fig. 12. The same holds true for the angle between the parietal and interparietal bones (5 4 3, graph U1) and between the occipital and interparietal bones (6 5 4, graph T3). It is worth noticing the serial changes in shape, as well as the inter-group differences at the individual stages, reproduced in figs. 11 and 12 and given numerical value as ratios of the total length of the components

Exp. 2, Stage	1	-	3	4	wean	6	7	8	9
Post-concep. Days	23	30	36	43	51	62	142	300	500



of the vault and their projected length (table 60). The ratio of height over length of the cranium $\frac{4\ 7}{2\ 5}$ is also given and compared between the groups (table 60).

For a detailed study of the relationships between the sphenoid bone and the various construction lines in the vault, reference is made to the angles 1 2 5, 7 8/1 2, (graphs N3, e1, R3, j2) and their combinations. Here, too, the shape changes are apparent. The angle 7 8/6 2 (graphs S2, i3) gives further expression to the growth behaviour.

Finally it should be remarked that the individual presentation of the extremes of each group, and of the rats 9 and 60, offers many interesting features in the aspects discussed above.

The estimate of the area of the total cranial vault (2 3 4 5 6 7 8 9 2, graphs B1, g1) at day 500 was correlated with the cranial capacity measured by mercury (see section D, page 36). This was found to be significant: $p < 0.005$ ($H_0: r = 0$) $r = +0.6238$ and $r = +0.6475$ for the small- and large-litter rats, respectively. This was considered to offer adequate support for the assumption that the area measured could be regarded as representative of the cranial capacity and, by expansion of this concept, of the cranial contents.

CRANIAL BASE

The cranial base consists of the basi-occiput, the body of the sphenoid bone, the presphenoid bone, and the cribriform plate of the ethmoid embraced by the frontal bone. This is embodied in the points 7, 8, 9, and 2, omitting the basi-occiput due to the uncertainty of locating basion.

In this subsection, the size and form of the cranial base and its relation to the surrounding structures will be discussed.

Findings

Table IV gives a survey of the information presented.

Table IV CRANIAL BASE DATA LOCATION EXPERIMENT 2

										Angles								
Quantity	2 7	2 8	2 9	3 9	4 7	4 8	7 8	7 9	8 9	7 8 9	8 9 2	4 7 8	7 8/1 9	7 8/6 1	7 8/6 2	7 8/1 2	6 5/7 8	5 6 3
Table Longit. Incr.	18	19	20	25	27		29			46	45	43	47	49	50	48	41	38
Graph Means Log-Log	•			•	•		•	•										
Graph Means	F1			H3	I3	J1	K1	K2	L1	Q3	A2	V2	R2	S1	S2	R3	S3	
Graph Individuals					c1	c2	c3			f1			j1	j3	i3	j2		

Analysis

The cranial base exhibits a pattern of growth which is related to that of the majority of skull dimensions, growing fairly evenly over the period studied as is shown by the form of the graphs of the relevant lengths (e.g. 7 8, 8 9, 7 9, 2 7, graphs K1, L1, K2, F1). This is in concord with the cranial vault length measurements and in contrast to the early increase and subsequent rapid flattening-out exhibited by the cranial vault height (4 7, 3 9, graphs I3, H3).

The angle between the sphenoid and the presphenoid (angles 7 8 9, graph Q3) decreases with age, and that between the presphenoid and the cribiform plate (8 9 2, graph A2) increases after stage 4.

The relationship between the cranial base and the rest of the skull varies in some aspects.

The relationship with the cranial vault became remarkably constant as has been observed from the angle 7 8/6 2 in the previous subsection. This is not quite the case with the facial area, where angles relating rhinion to the sphenoid (e.g. 7 8/1 9, graph R2) show a sharp change to a peak at stage 4, followed by oscillation. The same phenomenon is found in the relationship between the long axis of the whole skull and the sphenoid bone (7 8/6 1, graph S1).

Behaviour between the groups differs significantly both in size and in many angular changes. The small-litter rats generally show larger actual dimensions from stage 3 on, with the exception that no significant differences exist in the dimension 2 8, 8 9 and 2 9 at stage 9. From this it may be concluded that the part of the cranial base anterior to the sphenoid bone, and that is the one most closely related to the facial structures, catches up more or less completely.

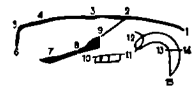
No significant difference was found in the angle 8 9 2 in any stage, but in 7 8 9 the small-litter rats showed a significantly smaller angle from stage 3 onwards.

The change in relationship between the long axis of the whole skull and the sphenoid bone (7 8/6 1, graph S1), deviates between the two groups in such a way that the increase in these angles is larger in the small- than in the large-litter group, so that rhinion becomes relatively more dorsally placed in those animals. This is supported by the behaviour of other angles relating to rhinion.

In the study of the graphs of the relevant quantities three types of change can be noticed: the rapid change of angles, with a saw-tooth form from after stage 3 (graph S1), the smooth and relatively gradual length changes (graph K1), and the sharp angular change to a maximum and back again as seen in the angle between the sphenoid and the supraoccipital bones (angles 6 5/7 8, graph S3). These forms are to be seen in examining the graphs as a whole, in other related dimensions.

This is obvious in the individual graphs. In graphs made from mean values of groups the individual variation is smoothed out. In individual longitudinal graphs an apparently larger

Exp. 2, Stage	1	–	3	4	wean	6	7	8	9
Post-concep. Days	23	30	36	43	51	62	142	300	500



variation can be expected, which sometimes makes the form of the graphs deviate considerably from the mean registration.

It is also worthwhile examining the behaviour of the cranial vault features relative to the cranial base. As an example, the parieto-interparietal suture moves anteriorly and posteriorly in relation to the sphenoid (angles 4 7/6 1, 4 7 8, graphs W1, V2).

In studying the graphs in combination, it can be seen that the angle 6 5/7 8 referred to above provides evidence that part of the changes in the caudal end of the vault are due to changes in relationship of the occipital and sphenoid bones. Its behaviour resembles that of the angles 4 7 8 and 5 6 3.

FACE

The face, as discussed here, embraces all the structures below and in front of the cranial base except the mandible. It is arbitrarily divided into two areas to represent the nasal and oral parts (areas 2 9 14 1 2, and 7 8 9 14 10 7, resp.). The dermal surface of the nasal bones is represented by the line 1 2, and the axis of the nose by the line 1 9. In this subsection, the size and form of the face and its components and its relation to the rest of the head is considered.

Findings

Table V gives the pertinent information regarding the data available.

Table V FACIAL DATA LOCATION EXPERIMENT 4

									Angles			Areas		
Quantity	1 2	1 7	1 14	2 7	2 10	2 11	7 14	11 13	7 8/1 9	7 8/1 2	1 2 5	1 2 9 8 7 10 14 1	2 9 14 1 2	7 8 9 14 10 7
Table Longit. Incr.	11	13	14	18	21	22	30	22	47	48	52	58	56	57
Graph Means Log-Log	•	•		•		•								
Graph Means	D1	D3	A3	F1	F2	F3	K3	M3	R2	R3	N3	B3	C1	C2
Graph Individuals	a1					b2		d1	j1	j2	e1	h2	g2	h1

Analysis

The general pattern of growth in size present in most of the dimensions already discussed is also found in the face, with however, another intensity (1 7, 2 7, 1 2, 2 11, 7 14, 11 13, graphs D3, F1, D1, F3, K3, M3).

The growth of the facial dimensions in height as well as in length, is initially much slower than that in the cranial measurements; but there is soon compensation, so that then the relative increments are higher than those found in the cranium. The same phenomenon is found in comparing the area measurements.

The angular changes with growth of the face in the rat reflect a growth forwards and upwards of the face relative to the cranial base (7 8/1 9, 7 8/1 2, tables 47, 48, graphs R2, R3).

The growth of the linear components (e.g. 1 7, 2 7, 1 2) is gradual and even. But its effect on the form of the head is to flatten the angle between the nose and the vault of the cranium (1 2 5, graphs N3, e1) very rapidly up till stage 4 as has been mentioned before. This is expected to be realised by a similar mechanism to that which compensates for the differences in growth of the individual bones, as has been indicated earlier, in the growth of the cranial vault alone (page 50).

Inter-group differences in the face are considerable. The small-litter rats have larger height and length dimensions. The same holds true for the areas measured.

It appears that the accelerated growth of the large-litter rats which enables them at least partially to catch-up in size, occurs later in the face than in the cranial structures.

Furthermore it is notable that the catch-up in height anteriorly seems to be more or less complete in contrast to that in the posterior face height (1 14 and 2 10, resp. tables 14, 21, graphs A3, F2).

The relationship between the face and cranium as previously stated, clearly displays differences that increase with time. The small-litter rats show more elevation of the face with respect to the cranial base and cranial vault than the large-litter rats (figs. 11 and 12).

It is necessary to realise in the study of the graphs in this region that in the earliest stages the points 10, 11, 12, 13, 15 are not present, and dimensions involving those points have no stage 1. This leads to a stretching out of the graph heights compared with those dimensions which do have a stage 1, giving an impression of more rapid growth which is false. Attention may be given to the behaviour of the areas in general (graphs B3, C1, C2) and in particular to the one involving points 2 9 14 1 2, in that the small-litter rats do not increase in this measurement between stages 8 and 9, whereas the other rats do. Furthermore it may be noticed that the angular changes are greater in the large-litter groups, in the angle 7 8/1 9 (graphs R2, j1) which joins rhinion to the cranial base. The difference is highly significant, after stage 6, and remains so. The angle 1 2 5, to which earlier reference has been made in this context, already shows that the angle is significantly larger in small-litter rats at stage 4, and it goes on to lose that difference by stage 9.

The dimensions of the jaws are also of interest in the consideration of the face, but the maxilla alone is considered here, in the next subsection. The mandible will be brought in as far as possible, in assembling the conclusions from this entire experiment.

DENTAL APPARATUS

In this subsection we will discuss the maxilla and its teeth. Some dimensions to be considered are:

Distances 2 11 and 11 13, which relate the M_1 to nasion and the incisor

Angles 1 2 11 and 6 3 11, which relate M_1 to the nose and the cranium, respectively

Angle 7 8/10 14, which relates the 'dental plane' with the direction of the endocranial surface of the sphenoid bone which represents a part of the cranial base.

Findings

The findings are presented as follows (table VI).

Table VI DENTAL APPARATUS DATA LOCATION EXPERIMENT 2

Quantity	Radius 12 14 15	2 11	10 11	11 13	13 14	14 15	Angles 1 2 11	6 3 11	7 8/14 10	1 2 14	1 2 10	6 3/10 14
Table Longit. Incr.	59	22	31	32	33		53	36	51			
Graph Means Log-Log	*	*										
Graph Means	C3	F3	M2	M3	N1	N2	O2	P3	R1	O3	O1	Q2
Graph Individuals	d3	b2		d1	d2		e2	e3	i2			i1

Analysis

The linear measurements recorded here can be distinguished in dental ones (10 11, 13 14, and radius 12 14 15) and distances that register other anatomical structures (2 11 and 11 13). Their behaviour differs to some extent.

The latter ones (2 11 and 11 13) show the general growth pattern as indicated before. Of the dental measurements, the total molar segment length (10 11) increases between stages 4 and 6 when M_3 erupts, and rather little thereafter. The measurements of the incisor width (13 14) and radius (12 14 15) conform more to the general growth pattern. This corresponds with the fact that they grow continuously.

The angular changes recording the orientation of the molar segment to the cranial vault (6 3 11) indicate a forward movement of the molar segment in relation to that structure. The dental plane inclines upward in relation to the sphenoid bone (7 8/10 14) till stage 4 and shows little change thereafter.

The inter-group differences for the linear dimensions are marked, for the molar width, for which a more or less constantly larger size in small-litter-size animals is maintained after stage 6. The width of the incisors shows a significant difference in all stages except the 7th one, where the catch-up seems to permit an approach to the small-litter-size dimensions. The radius of the upper incisors shows a significant difference in all stages including the last one.

It may be mentioned here that the form of curvature of the incisors is not a circle but a more complex spiral, for which a radius is insufficient description.

The other dimensions recorded in the face (2 11 and 11 13) are larger in all stages for the small-litter-size animals.

The group differences in position of the molar segment in relation to the cranial vault (angle 6 3 11, graphs P3, e3), indicate a relatively more caudal relation of the molar segment to the cranial vault in large-litter-size animals.

The orientation between the sphenoid bone and the dental plane (7 8/10 14) differs between the groups at all stages, the angle being larger in large-litter rats.

Examination of the graphs and tables shows that the manner in which the quantities behave varies in the time at which the greatest changes occur. This can be seen in the vertical closeness or otherwise of the points on the graph. For example, angle 7 8/14 10 changes rapidly in the beginning, but then comes more or less to a standstill. Both experimental groups follow the same patterns in this respect, but significantly deviating in most values.

Study of the incremental data tables brings out the fact that the large-litter rats grew significantly faster in the incisor dimension than the other group, generally after stages 4 or 6. The difference in increments reduced with time in all cases, after the initial spurt. This also occurred in the extra-gingival incisor length (graph N2, distance 14 15 (no table)) which is noteworthy because this length is supposed to be related to contact with the lower incisor, and the matching of attrition with eruption rate. It is here seen also to be related to the experimental grouping.

The angle 1 2 11 shows essentially no systematic difference between groups, nor does this situation change throughout the stages. Other associated angles such as 1 2 10, 1 2 14 show similar lack of difference. With reference to these angles, it should be remembered that the third molar erupts between stages 4 and 6, so two molars are in the distance 10 11 at stage 4 and three teeth are involved in this measurement from stage 6 on.

Yet this appears not to affect very much the angle subtended by the molar segment. This can be explained by a comparable increase in distance between the molar points and point 2. It is seen that the point 11 moves slightly forward relative to 1 2 as M_3 erupts, but in the following period retreats nearly to the original angular relation. The apparent forward movement is greater in the odd than the even group, judging by the superimpositions on line 7 14 in the outlines (see fig. 11). The angles 6 3 11 and 1 2 11 react in a dissimilar way, the former evenly, the latter oscillating.

It may be remarked here that the changes in these angles involving 1 2 are greatly influenced by the behaviour of the nasal bone. This displays a marked difference between the groups as indicated in the previous subsection. The remarks made here regarding the position of the molar teeth in relation in the nasal bone therefore are relative and may as well be interpreted as the result of a relatively more dorsal movement of the nose in the small-litter rats.

SKULL IN TOTO

When the regions discussed separately are combined and the skull as a whole is considered, further aspects show up. Of course this cannot be discussed

Exp. 2, Stage	1	-	3	4	wean	6	7	8	9
Post-concep. Days	23	30	36	43	51	62	142	300	500

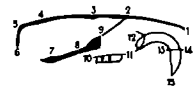


Table VII WHOLE SKULL DATA LOCATION EXPERIMENT 2

							Angles							Areas				
Quantity	1 2	1 6	1 7	2 6	2 11	3 9	1 2 5	6 3 1	5 6 1	4 7/ 6 1	7 8/ 6 1	7 8/ 1 2	7 8/ 6 2	2 9 14 12	2 3 9 2	1 2 9 8 7 10 14 1	2 3 4 5 6 7 8 9 2	7 8 9 14 10 7
Table Longit. Incr.	11	12	13	17	22	25	52	34	37	44	49	48	50	56	55	58	54	57
Graph Means Log-Log	*	*	*	*	*	*												
Graph Means	D1	D2	D3	E3	F3	H3	N3	P1	T1	W1	S1	R3	S2	C1	B2	B3	B1	C2
Graphs Individuals	a1	a2		b1	b2		e1				j3	j2	i3	g2	g3	h2	g1	h1

properly without repeating some of the information presented before (table VII).

The face begins as a relatively small part of the head, so that at birth, as can easily be calculated from the tables, the nasal length (1 2) is about 33% of the cranial length (2 6). At stage 6 it has become about 50% and by stage 9 is some 65% of the cranial length measured between points 2 and 6. Taking the areas for the cranium (2 3 4 5 6 7 8 9 2) and face (1 2 9 8 7 10 14 1) that we have utilised, the first comparison is possible at stage 3, when the facial area is about 50% of the cranial. By stage 6 it is almost 90%, and at stage 9 is over 125% of the so-called cranial area.

The length of the skull overall, 1 6, behaves according to the general pattern. It grows smoothly and at quite a high rate. The nasal length 1 2 grows less quickly in the early stages but keeps on longer. The relative increments are moreover nearly double those of the cranial length 2 6. The difference between the groups in nasal length is smaller than in cranial length, in all stages.

The posterior cranial height (4 7) grew in an unusual way, as was discussed earlier, without showing any difference between both groups. The anterior cranial height (3 9) reacted similarly in the pattern of its growth, but showed in general a significantly larger dimension in the small-litter rats after stage 4. The ventral components of the cranium grew more than did the dorsal ones and a rotation upwards of the face ensued, augmented by the differential growth occurring somewhat more in the tooth-bearing parts of the face than at a higher level, as exemplified by the difference between the increments in the lengths rhinion-synchondrosis (1 7) and rhinion-nasion (1 2).

As this rotation, as it affects the angle between the dermal surface of the nasal bones and the axes of the cranium (1 2 5, 7 8/1 2, 7 8/6 2), was more or less complete by stage 4, the remaining changes in the form of the skull may be attributed to the alterations in the caudal part of the cranium, particularly in the region of the interparietal-parietal suture. The angle 5 4 3, which is also significantly smaller in the large-litter group, gives the necessary evidence to support this suggestion.

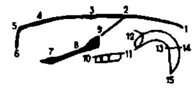
Study of the velocity and acceleration data derived from the fitted curves for various dimensions (those used in figs. 13–16), indicated that the instantaneous growth velocity at the time of measurement at the various stages showed few significant differences between the groups. Those that were showed that the large-litter rats were growing faster except in the case of stage 3 when the dimension 7 14 (spheno-occipital synchondrosis to supradentale) in the small-litter rats was growing faster. This last result was also found in the deceleration in growth rate exhibited by 7 14 in the same stage. The sphenoid bone length (7 8) in stage 4 did the same. Significant differences between the groups in deceleration were less often seen than in velocity, and apart from those two just mentioned, the large-litter group showed more rapid deceleration throughout.

Discussion

Although rats are commonly used laboratory animals and although there is a fund of literature on measurements of many kinds, there has been little systematic study of form changes. Mortimer (1937) had early realised the value of an x-ray study of rats, and used this method on cross-sectional material for study of the effects of hormones. He noticed differences in form.

Spence (1940) employed the techniques of Broadbent to make serial studies of rats by standardised cephalometric x-ray pictures. Asling and Frank (1963) published a description of a craniostat for rats, comparable in performance to the one developed by the orthodontic department in Nijmegen and used in our study. They further employed longitudinal x-ray material to study the effects on growth subsequent to hypophysectomy. They investigated shape changes, further work by Wright et al (1967) being a development of this. Diamond et al (1965) have also followed their example. But that work was of a limited nature, with attention focussed on rather broad concepts. They were able to show changes in proportions in their animals as a consequence of experimental intervention. Moore (1966) made a longitudinal normal growth x-ray study of rats at monthly intervals between 1 and 5 months of age, drawing attention to the changes in skull proportions over that period. Du Brul and Laskin (1961) followed rats from which the spheno-occipital synchondrosis was removed at various ages. They found similar results as in Asling's animals that had also been hypophysectomized. Reisenfeld (1967) studied the head form of rats suffering starvation, but not with x-rays. Studies such as those of McCance (1960) and others have brought shape changes to the attention of the student, but their measurements have been on a cross-sectional basis as far as

Exp. 2, Stage	1	–	3	4	wean	6	7	8	9
Post-concep. Days	23	30	36	43	51	62	142	300	500



shape in concerned. Of course, the study of form has been of age-long interest, but an investigation resembling the present one has not come to light.

The present work confirms the findings of the above workers, that different forms of interference with the growth processes of an animal may be followed by distortion of the form of the head. It has been found that the normal rats had more elongated and straighter heads than experimental animals, more or less regardless of the nature of the intervention. This effect in hypophysectomy is hardly surprising, whether from the loss of the pituitary or from the operative procedures. However, underfeeding and other environmental changes are not so easily seen to be likely means of changing skull shape.

The details of form changes in the rat skull revealed by this work, correspond with the limited information already available. The catch-up in growth that has been mentioned before (Chapter I), was found to occur in practically every dimension in the repressed rats. The notable and understandable exception was the non-growing molars. The relatively unaffected posterior cranial height displayed very little catch-up, and it is the question as to whether or not this was the result of the fact that the height had not departed enough from that of the other group to make an acceleration of growth necessary. But what mechanism recognises achievement of 'normality'? This resistance of the skull height to change has been frequently remarked upon in the literature, e.g. by the workers mentioned above. It is not possible to say from this experiment why the changes have occurred as they have, but a study of the evidence available may indicate in which region a more specific interference may produce a like result.

The principal difficulty in any such study of change is in relating the changes to a logical base-line. Every change is relative, so that it is impossible to state in this type of investigation, what it is that changes, and what stands still. We can however clearly state that differences exist, even if we cannot say exactly in which direction the experimental effect is acting.

Conclusions

The investigation of the growth of the skulls by cephalometric x-rays, has shown a basic pattern of growth which is similar in both experimental groups.

The bulbous skull of the newborn rat elongates and flattens with age, the face becoming a greater proportion of the whole while growing upwards and forwards with respect to the cranium.

The cranium itself flattens by alteration of the relationship of the components of the vault, and the occiput becomes first more upright with respect to the head axis, and then relapses. The cranial base shows a decrease in the angle between the sphenoid and presphenoid bones 7 8 9, while the angle between the presphenoid and the anterior cranial base 8 9 2 eventually flattens.

Concurrent with these changes it is noteworthy that the sphenoid bone maintains a constant relationship with the cranial axis after stage 3 (angle 7 8/6 2).

Some parts of the skull grow rapidly in the initial period after birth and then change little more. Other parts grow gradually and evenly, and although slowing down to a very slight growth rate, nevertheless continue to grow at least to the end of the period studied. The early changes occur in those dimensions concerned with the cranial height, the orientation of the cranium and rhinion, the behaviour of inion, and to some degree, the angle between the sphenoid and presphenoid bones. The remaining dimensions change later. In general the cranium grows more rapidly in the beginning and the face grows more in the later periods studied. This leads to the changes in proportion mentioned above. A few angular registrations show peaks at stages 4 or 6 e.g. those concerned in the eruption of the third molar. Other 'peaky' registrations are generally related in some way to the posterior part of the cranial vault.

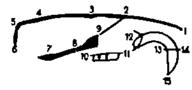
The effect of the experiment on this pattern was shown to be the retardation of growth of the large-litter rats in such a way that most dimensions at stages 4 or 6 show a significantly greater size in the small-litter rats. But not only is the size less in the large-litter rats, they have a different form. There are significant differences in the relationship of the parts, so that the large-litter rats have a less elongated and a more rounded head, both in the shape of the cranial vault and in the more ventral relationship of the nose to the cranium. These differences diminish in time and are not present any more at stage 9 in the angle 1 2 5, for instance. The cranial base however is less curved, posteriorly, in the large-litter rats (angle 7 8 9).

No difference between the two groups is found in the posterior cranial vault height during the whole period of observation. The elimination of the condition of living in a large litter, occurring at weaning, appears to permit a recovery of the growth potential of many but by no means the greater proportion of the dimensions.

Those dimensions which show no significant difference at stage 9 are those concerned with the cranial base, angles related to the line rhinion-nasion, the relationship of the molars and incisors to the face, and the features of the posterior part of the calvaria.

Reference to the mandible is pertinent at this point. It was not possible to relate the mandible directly to the rest of the head. No method of securing a reproducible jaw relationship was known. But a comparison of size and changes in size can be made by comparing the length from the molars to the incisal edge in the mandible (7 10 section H) with the distance from molar to palatal of incisors (11 13 Section I) in the maxilla, at stages 6 and 7.

Exp. 2, Stage	1	–	3	4	wean	6	7	8	9
Post-concep. Days	23	30	36	43	51	62	142	300	500



This reveals very close similarity not only in these dimensions but also in their increments, and in the degree of inter-group differences.

The mandible of the large-litter rats moreover, conforms in shape with that of the small-litter rats at stage 7. This conformity of shape, while being susceptible to size differences, is of interest. It is also apparent that the catch-up mechanism is operating in the mandible in favour of the large-litter rats.

It is possible to show very highly significant differences in the increments of most dimensions, in both the mandible and the skull proper. What is of great importance is that before stages 4 or 6 the difference is generally in favour of the small-litter rats, but after that time the difference is decidedly in favour of the large-litter rats. *It is this extraordinary acceleration that is responsible for the possibility to restore some or most of the missing growth, in many dimensions.*

Summary

Litter-size extremes of 3 or 17 rats per mother were imposed from birth to weaning at 51 days after conception, on paired randomised male Wistar rats conceived at the same time. Two such experimental groups of about 30 rats each were studied longitudinally till 500 days post-conception.

The animals of the large-litter group became smaller in size than the others. The rats in the large litters were less well-cared for and had the appearance of being underfed. Weight records showed an inter-group difference (large-litter rats $<$ small-litter rats) that was significant at day 25 post-conception (2 days after birth). This difference persisted throughout the period of the experiment, but reduced gradually after weaning.

Measurement of tail and body length and a study of the ratio between the two, led to the conclusion that the difference in experimental environments induces a difference in the growth of the body of the rat which is greater than occurs in the tail. This difference in growth also becomes less with time.

Histological study showed no difference, neither in the structures of the skull nor the adrenal glands.

But although no significant difference was found in comparing the weight of the adrenals in relation to body weight, they were, however, significantly smaller in absolute weight in the large-litter group than in the other.

A similar observation was made regarding the cranial capacity; however, here a positive relationship between body weight and cranial capacity in the large-litter rats was detectable.

Maxillary molar size as measured on lateral cephalometric x-ray pictures taken 82 days after conception showed a significantly smaller dimension in the

large-litter rats, for total molar segment length, and for mesio-distal crown width of the second and third molars.

By the same means the growth of the mandible was studied from computations of a large number of angles and distances between 10 points. It was found that significant inter-group differences in size could be discerned at both post-conception ages studied (62 and 142 days). In general, shape showed no differences. The differences in size were less by the time the last-mentioned age had been reached.

The skull itself was studied longitudinally from 36 to 500 days post-conception using 15 points. From this it was seen that the face developed later than the cranium. The shape of the skull changed from bulbous to elongated, by relatively more growth in nose length combined with a reorientation of the parts of the cranial vault to each other, and of the nasal bones to the cranium. This was seen in the elevation of the face.

Inter-group differences could be seen in the way in which these changes occurred, resulting in the large-litter rats having a more bulbous final form. Dimensions in the face showed more ability to recover, as was evidenced by a reduction of the differences there compared with those in the cranium. A notable phenomenon observed was the way in which the cranial height was the least affected dimension. The pertinent increments displayed by the large-litter rats were significantly larger than those of the remainder.

It was felt that more information on the phenomenon of accelerated and retarded growth could be obtained by studying animals in which the experimental conditions were changed over at an even earlier age. A study of such a type is presented in the next chapter.

CHAPTER VI

REVERSING EXTREMES OF LITTER-SIZE AT DAY 30: EXPERIMENT 4

In this series of experiments, identified as Experiment 4, the experimental conditions were reversed on day 30 (7 days post-natally) with the object of seeing what effect the shorter duration of living on a low plane of nutrition would have on rats put in large litters 2 days after birth, and in optimal conditions 5 days later. Furthermore by reversing the conditions it would be seen what effect later imposition of a low plane of nutrition would have on the rats initially reared in small litters. It was also hoped that the expected change in growth rates would show up as differences in the time at which the various measurements of the two experimental groups crossed over.

With the information presented in the previous chapter in mind it will be realised that at day 30 the rats initially in small litters would be notably bigger than those from large litters, but upon imposition of the reversed conditions the growth of the larger rats would be retarded and of the smaller accelerated, so that at a particular future moment both groups would be the same weight and similar size. Were all dimensions affected to the same degree, that moment would be the same for all dimensions. Were it not so, then the identity of the various dimensions would occur at different times. Unfortunately this is not as simple as it seems, since in using averaged measurements individual variations may be expected, causing a dispersion that makes registration of the crossover points rather uncertain. Therefore in addition animals are compared at random individually.

GENERAL INFORMATION

Examine figures 5 and 6 for a schematic illustration of the experimental design.

Rats conceived on the night of 3/4 April 1966 in the same manner as in Experiment 2, were born on day 23. The males were sexed and numbered and weighed shortly after birth and then replaced in their original litters. On day 25 they were removed, weighed and separated in two groups according to their odd or even numbers. The odd numbered rats were divided into two large litters of 18 each, and the even numbered rats were divided into 12 litters of

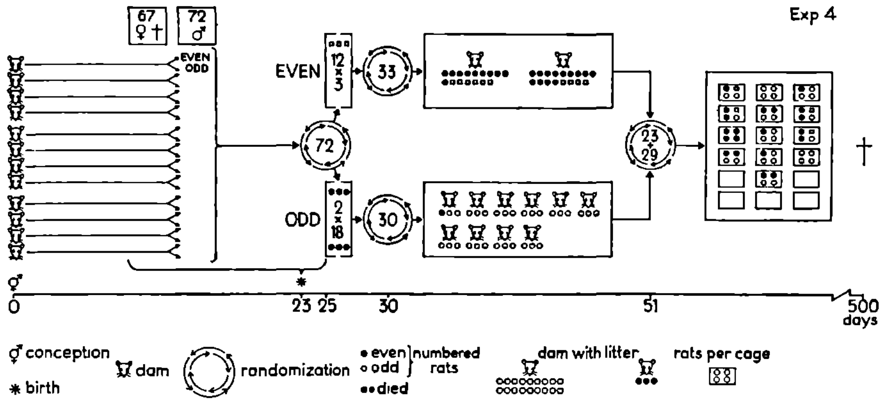


Fig. 5. Schematic illustration of the design of Experiment 4.

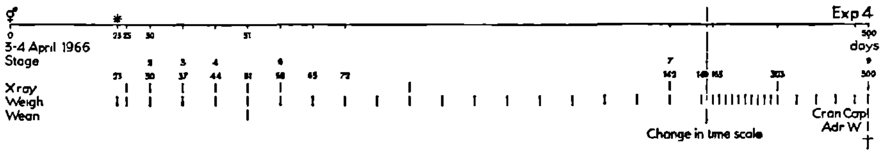


Fig. 6. Experiment 4. Timing of stages and records. Note the slight differences in the days on which specific stages occur, compared with Experiment 2. Again all x-ray records were not used.

three. This redistribution was done according to tables of random numbers. The females were discarded. At day 25 the rats were also subjected to lateral cephalometric x-rays. On day 30 the rats were redistributed again so that now the odd-numbered rats were split in litters of 3, and the others were gathered in two large litters, given to the same two mothers who had fostered the original large litters.

Changes in the numbers due to deaths can also be seen in figure 5. Upon weaning at day 51, the rats were redistributed again, into cages with four rats per cage. No attention was paid to the original grouping of the rats, but allocation was entirely according to random numbering, with the odd and the even numbers mixed. Food and water were available ad libitum after weaning. On day 500 the rats were sacrificed, the cranial contents were aspirated and the suprarenal glands were removed and kept, with the head, in 4% formalin. Formalin perfusion was not used, to facilitate suction of the cranial contents.

Compared with Experiment 2, the mortality in this series was considerably greater, being notably larger in the large litters. Again the anaesthetic was the principle cause of death, but apparently the experimental conditions made the smaller rats more likely to succumb.

a. SUPERFICIAL OBSERVATIONS

Nothing was seen in these animals to distinguish them from the previous ones. They were less aggressive however, and this is attributed to the absence of the rather firm handling needed for tail and body length measurements in the previous series.

b. WEIGHT

Method

Weighing was done on days 23, 25, 30 and every 7 days till day 163. The next weighing was on day 177 and every 14 days till day 303, when the period was changed to 42 days. The last weighing was done when the rats were killed on day 500. As mentioned before, the timing of weighing is in this respect the only variable between Experiment 2 and 4 in the collection of the weight data.

Findings

Figures 8 and 10 should be referred to again for the evaluation of the course of the weight gains within this series and for comparison with the previous ones. Table 2 gives the numerical data.

Analysis

It is clear that the experimental groups remained closely similar till day 25, when the extremes of litter size were imposed. At day 30, the day of changing over, the rats which had been in large litters were significantly smaller than those till then in small litters. On day 37, the same rats which had been smaller had gained about 10 grams and were now significantly larger than those of the other group who had gained 5 grams in the intervening week. In the week thereafter respectively 15 grams and again 5 grams were added. The now favoured rats nearly doubled their weight in 7 days time. Interpolation indicates that both groups must have had similar weights at about day 35.

Discussion

No information has been discovered in the literature pertaining to a comparable experiment. Comparison with the previous experiment (figs. 8 and 10) can only be made in broad lines, and not in details because there are differences in the whole set-up. However it seems likely ($p < 0.001$ $H_o: x_e - x_o = 0$) that the weight gain of the even-numbered rats in Experiment 4 is more depressed by the changed conditions on day 30, than was the case with the rats of Experiment 2 which had been in a large litter from the day of birth. Between days 30 and 44 the former gained about 10 grams compared with the gain of say 15 grams for the small rats of experiment 2. This does suggest that the shift from high to low planes of nutrition is a more severe experience than continually living on a low plane. Both comparable groups in this series finish with a smaller weight than the groups of the previous experiment (H_o: $p < 0.001$), but as remarked above, it is not possible to say definitely if that is due to the experiment. On the other hand, the final weights are much closer together than was the case in Experiment 2.

Conclusions

The divergence of the two groups is such that at day 30 the small (odd-numbered) rats are about 75% of the weight of the larger. In contrast, at day 100 the even-numbered rats are about 86% of the weight of the odd-numbered ones. The greatest divergence occurs at about day 51, when the percentage weight of the small rats compared with the big ones (odd-numbered, initially large-litter rats) is only about 60%. This seems to occur rather later than in the first series.

Reversal of the experimental conditions at day 30 leads to a crossing over of the paths of the weight records of the groups at about day 35. The difference between the experimental groups in the weights of the animals in general decreases in time but is still significant at day 500. Comparison with the previous experiment indicates that there is a greater degree of recovery here, in relation to the other group. However the upper group probably is depressed and that makes this comparison of limited value.

C. CRANIAL CAPACITY

Method

Aspiration of the cranial contents was done prior to fixing the tissues. Otherwise the method was the same as that employed in the previous experimental series.

Findings

The table 8 gives the means and standard deviations of the measurements, and the correlation between body weight and cranial capacity, treating the experimental groups separately. The results of Student's 't'-test are given between the group cranial capacities and for the correlations after applying Fisher's z transformation.

Analysis

There is a significant difference between the groups in the values for the cranial capacities. The larger rats have the larger cranial capacity, when taken as groups. However, the correlation between weight and cranial capacity is not significant in the case of the rats which were put into large litters at day 30, although it is significant in the other group. The difference in the correlations is not found to be significant. This may be related to the rather small number of subjects and the inherent difficulty of testing the significance of a correlation coefficient that is close to zero.

Discussion

There is a lack of comparable data. As previously explained, it is also rash to compare the results of this and the previous experiment. Taking that risk, the application of Student's t-test indicates that there is a difference in the final cranial capacity of the comparable groups of rats of the two series ($H_0: P < 0.001$). The smaller rats of the first series had an average capacity of about 27.7 gm Hg, compared with the 25.6 gm of the second series small rats. The average values for large rats of the series were about 29 and 26.5 gm Hg, respectively. The inter-series difference between the cranial capacities found in Experiments 2 and 4, is of similar proportions ($H_0: p > 0.20$) in both the case of the small rats and the large rats. This is not so in their body weights, the inter-series difference between the final weights of the large rats from the experiments being about 60 gm and of the small, 20 gm. It means that a similar degree of catch-up occurred in the cranial capacities in both experiments even though a more complete catch-up in body weight occurred in Experiment 4 than in Experiment 2.

The findings in Experiment 7 throw more light on this phenomenon. Therefore this will be further discussed at that time.

Conclusions

The experimental conditions led to a difference in the cranial capacity of the two groups, those rats subjected to deprivation between days 25 and 30 having larger capacities than those deprived between days 30 and 51. The difference between the groups was approximately the same in this as in the previous experiment although the absolute values for cranial capacity were smaller in this experiment.

There was no strong relationship between body weight and cranial capacity.

d. ADRENAL GLAND WEIGHT

Method

The only difference from the previous experiment was that there was no formalin perfusion used for fixation.

Findings

Table 8 gives the weights of the adrenal glands of both groups as means and standard deviations. The correlation between body weight and adrenal weight is also given. The weight is also expressed as mg/100 gm body weight.

Analysis

There is no significant difference between the weights of the adrenal glands of the experimental groups. Although a significant correlation is found between adrenal weight and body weight in the odd-numbered group, that is, the group living in small litters after day 30, this was not the case in the other group and this may be related to the difference in sample numbers.

Discussion

The reaction to underfeeding mentioned in the previous chapter under this heading, where relative enlargement is found in the adrenals, would seem to have occurred in this experiment. It is not possible to say definitely however if that is indeed the case, since the relative change may be not an enlargement of one group, but a decrease in size of the other.

Conclusions

Under the experimental conditions there was no significant difference found in the weight of the adrenal glands when compared between groups as absolute values. A positive correlation between body weight and adrenal weight was found in the initially large-litter rats. This was not the case for the other group.

e. HISTOLOGY

Method

This was identical to the last experiment except for the previously mentioned difference in fixing.

Findings

No differences were found from what would normally be expected in rats of this age.

f. MOLAR SIZE

Method

Out of lateral skull x-rays taken on days 37, 44, 51 and 58 the best pictures of the teeth were chosen. This meant that some rats were represented on more than one occasion.

Upper molars were measured by the same means as in Experiment 2. In this experiment the lower molars were recorded in the same way in addition. In both instances all three molars could only be measured on the last two occasions.

When one rat was measured more than once, the mean values of the measurements were used.

Findings

The data are presented in table 9.

Analysis

The smallness of the samples is regrettable. This reflects the mortality and also the accidental occurrence of a lesser number of good pictures of the teeth of the even-numbered rats.

The findings indicate that the mesio-distal length of the maxillary M_2 and the mandibular M_1 and M_2 are significantly larger in the even than in the odd animals. The M_3 in the maxilla exhibits no difference, and that in the mandible shows a difference which is not significant at the arbitrary 5% level, but which is interesting in that it is a difference in the opposite direction to the difference in M_1 and M_2. For in contrast to the other teeth the lower M_3 seems to be larger in the odd-numbered rats, that is, in the rats living in optimum conditions after the change-over at day 30.

Discussion

The probability that the third molars are larger in the odd-numbered rats, is not surprising. The second molars would be in their most rapid period of growth around day 25 or day 26 and being completed in their mesio-distal dimensions at day 35 (Schour and Massler 1949, Paynter and Hunt 1964). The conditions imposed between days 25 and 30 could be expected to be predominant in influencing the size of the second molars, forming rapidly in that period, resulting in smaller molars in the animals then in large litters i.e. the odd-numbered litters. The third molars however are growing fastest just before day 43 and would then be expected to be most susceptible to the growth influences in the period from 30 to 51 days. Hence a reduction in growth rate of that tooth bud in the odd-numbered rats in the initial experimental conditions, may be expected to be compensated for by the later improvement in conditions. The opposite is to be expected for the even-numbered rats.

It does not appear from the total data that the final difference developed in tooth size is as great as that in body weight. This could of course be accounted for by the fact that weight changes are continuous, while the growth of the molars is quickly coming to completion. An extension of this comparison of continuous and discontinuous phenomena will later be seen in looking at the incisor growth.

Conclusions

The experimental conditions produced a difference between the groups in the mesio-distal lengths of the rat molars, according to the conditions existing at the times the respective teeth were developing. Good conditions produced larger teeth in comparison to poor circumstances.

g. SKULL

Introduction

The investigation of the skull reported in this chapter is based on the same features as in the Experiment 2. It will be possible here to indicate also the differences seen between the two series, most particularly in the way the new experimental design affected the inter-group differences. Attention will only be drawn to general growth pattern where it is of particular interest.

The findings are presented as far as possible adjacent to the similar findings of the previous experiment. Due to the absence of stage 8 in this series, the last stage graphed in the mean graphs had to be stage 7 (day 142). This affects the slope of the graphs slightly, making them in general steeper than those of Experiment 2. Of more importance however, is that one should note the vertical scale. The values seldom coincide with those of the earlier experiment. Comparison of pattern, not size, was the prime purpose. Due to the fact that stages 8 and 9 are not presented in the mean graphs a comparable overall visual impression between the series cannot so easily be gained. Therefore in the evaluation of the data presented in the following part of this chapter the tables will be of more importance. It will be seen that the graphs of individuals (which include stage 9) are indeed comparable in every respect, as are the log-log graphs and the mean outlines (figs. 13 to 16 and 11 and 12).

The skull will be dealt with in the same parts as before: cranial vault, cranial base, face and dental apparatus.

Method

Lateral cephalometric x-rays taken on days 30, 37, 44, 58, 142, and 500 post-conception, were used, in combination with those taken on day 23 in the following Experiment 7. The handling of the films was precisely as in the previous experiment.

CRANIAL VAULT

Findings

The findings are to be found as indicated in table VIII.

Table VIII CRANIAL VAULT DATA LOCATION **EXPERIMENT 4**

										Angles							Areas	
Quantity	23	25	26	34	37	39	45	47	57	125	543	563	654	78/12	78/62	63/47	2392	23456 7892
Table Longit. Incr.	15	16	17	23	24	25	26	27	28	52	40	58	39	48	50	35	55	54
Graph Means Log-Log			*		*	*		*	*									
Graph Means	E4	E5	E3	G4	G5	H6	I4	I6	J6	N6	U4	T5	T6	R6	S5	Q4	B5	B4
Graph Individuals		ab	b4	b6				c4		e4	f6		f5	j5	i6	h6	g6	g4

Analysis

The general pattern of the changes in the cranial vault closely follows that of the previous experiment. Here too the growth is most prolific in the early weeks of life, particularly in the height dimensions, and the shape of the cranial vault becomes less bulbous (table 60). It can be seen how the odd-numbered rats had the most bulbous vaults initially, but after reversal of conditions the even numbered rats (now in large litters) had the most bulbous vaults. Small-litter rats in stages 4 and 6 show a significant ($p < 0.05$) inter-series difference in the index 2 5/4 7, having more elongated crania in Experiment 2. The vault shape 2 5/ (2 3 + 3 4 + 4 5) was rounder in Experiment 2 in both groups in all stages except stage 9 where no significant difference was seen in small-litter rats.

In actual size, the both groups are rather smaller ($H_0 : p < 0.05$) in this series though height dimensions tend to show no difference. There is some variation according to group and stage but the general difference is significant.

The angular relationship of the cranium with the sphenoid bone is remarkably constant after stage 3 as in the earlier experiment, even showing the same crossing over in the graphs between stages 6 and 7 (angle 7 8/6 2, graph S5).

The obvious distinction between the two series is found in the behaviour of inter-group differences. In the first place there is a significant reversal in the sizes after the experimental conditions changed at stage 2. This alteration in growth does occur in every dimension, but in a few it is not significant. The difference in group values for linear dimensions is greatest around stage 6, but some dimensions show differences at other stages.

The second inter-series distinction is that in all tabulated linear measurements except the ones representing the caudal vault height (4 7, 5 7) no significant inter-group differences remain by stage 9. In contradistinction, in the first experiment, there was only a minority of dimensions where no significant differences could be found in the last stage. No angle (among those we have computed) shows a significant difference between the groups at stage 9 in Experiment 4.

The observations on the relationship between the cranial vault and the rest of the head are only different in that there is now no longer a noticeable difference between the groups in this respect. The value of angles expressing this relationship is generally very similar to that of Experiment 2.

The mean rat outlines may illustrate the points indicated above (figs. 11 and 12).

A study of the graphs and tables requires care, as previously pointed out, due to the necessary but unusual scales on the y axes. It would be wise first to examine the graphs of individuals, where it soon becomes apparent that there is in general a more regular behaviour in these graphs than in the previous experiment, but that otherwise the pattern is similar.

Regarding the graphs of the mean values it may be noticed that, in these too, the manner in which the rats initially in small litters cross to the lower side of the others after stage 2 is well illustrated in e g. angle 5 4 3, or distance 11 13. The catch-up is also readily visible

Overall, the graphs confirm that the mean pattern is a reasonable portrayal of the individual behaviour in both groups in both series.

Those dimensions whose characteristics bear close study are again those connected with cranial height. The rare lengths showing a significant inter-group difference at stage 9, are 4 7 and 5 7 (tables 27, 28) 5 7 Is especially interesting because it shows a significant difference at all stages except stages 2 and 1 4 7, On the other hand is interesting because although it showed no significant differences in the previous experiment, it now shows significant differences in the later stages where most other dimensions are showing similarity But a more extreme example in the other direction is found in 4 5, which never shows a significant difference In Experiment 2 it at least showed a significant difference at stages 3 and 4. The similarity of the graphs between both series is most impressive.

It is interesting that the angles showing differences at stages 2, 4 and 6 (angles 5 6 1, 5 4 3, graphs T4, U4) are concerned with the cranial vault. 6 5 4 Is here anomalous since it is at no time significantly different between groups.

Attention should be paid to the areas measured It will be seen that one of them exhibits the influence of the litter sizes rather well. The area representing the total cranial area (2 3 4 5 6 7 8 9 2, graph B4) is significantly smaller for the even-numbered (initially in small litters) animals at stage 2. After the reversal of the experimental conditions the difference is the other way around and continues to be so. To a certain extent the same is found for the smaller olfactory area (2 3 9 2, graph B5), however here the difference was not significant at stage 2 nor at stage 9.

The incremental differences are also worth study in the tables. It is not till between stages 6 and 7 that the increments of the even-numbered rats (initially in small litters) become significantly greater than those of the other group, and this of course influences the time when the differences between the groups are eliminated. This phenomenon can be seen in many graphs also In Experiment 2, this achievement of significantly greater increments in the smaller rats occurred after stages 4 or 6 and was not so dramatic a change. The double change in the relationship between the growth of the groups and the very high value of significance of the differences, is a phenomenon that demands attention in Experiment 4

Length 4 5 (graph I4, table 26), is unusual in the above respect, and repays study. Angle 6 5 4 illustrates as do several other quantities how significantly different increments can so balance out in the conditions of this experiment that the result in the actual measurement is no significant difference (table 39). Be careful particularly in studying angles that the t-values with a negative sign are associated not only with the reversal of the differences between odd and even rats, but also with whether or not the increment is in fact a decrement. Other details of the cranial vault can be examined as suggested in the previous chapter, when it will be found that the behaviour of animals in the same circumstances corresponds in character even if it differs in magnitude. The posterior end of the cranium seems to differ in form between these experiments.

The correlation between measured area and cranial capacity remained quite high in this experiment also, r being 0.5984 and 0.7520 for small-litter and large-litter rats respectively at day 500.

Findings

Table IX gives a survey of the information presented.

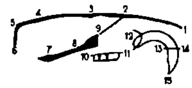
Table IX CRANIAL BASE DATA LOCATION EXPERIMENT 4

Quantity	2 7	2 8	2 9	3 9	4 7	4 8	7 8	7 9	8 9	Angles 7 8 9	8 9 2	4 7 8	7 8/1 9	7 8/6 1	7 8/6 2	7 8/1 2	6 5/7 8	5 6 3
Table Longit. Incr.	18	19	20	25	27		29			46	45	43	47	49	50	48	41	38
Graph Means Log-Log	*			*	*		*	*										
Graph Means	F4			H6	I6	J4	K4	K5	L4	Q6	A5	V5	R5	S4	S5	R6	S6	
Graph Individuals					c4	c5	c6			f4			j4	j6	i6	j5		

Analysis

The growth of the cranial base proceeds as in the earlier experiment. It follows the general growth pattern, of gradual even increases. In actual comparison of the cranial base lengths (7 8, 2 7, 2 8, 2 9) and angles (7 8 9, 8 9 2) between Experiments 2 and 4, the latter seem to be smaller ($p < 0.001$), and the angle 8 9 2 (graph A5) in the anterior part is also smaller ($p < 0.001$) after stage 4. But comparison between the groups shows no significant difference at stage 9 in total length or any other cranial base dimension although it is apparent that the sphenoid in the odd-numbered rats (initially large litters) is significantly larger than those of the other group after stage 3, but that this difference has disappeared between stages 7 and 9 (7 8, graphs K4, c6, table 29). There is at no time a significant difference between the groups, in the angle 8 9 2, but the angle 7 8 9 does show a difference at stage 6. The size of the angle fits the situation in the Experiment 2, where the larger animals (i.e. from small litters) had smaller angles between the sphenoid and presphenoid bones than did the less well cared for rats. In this experiment the smaller angle at stage 6 is seen in the mean outlines of the odd-numbered rats. One should note that in Experiment 2, the angle 8 9 2 showed no significant inter-group differences, but 7 8 9 showed differences at stages 3, 6, 7, and 8.

The relationship of the cranial base to the rest of the head is only different from the previous experiment in the extent to which no significant differences are now seen between the groups (angles 7 8/1 9, 7 8/6 1, graphs R5, j4, S4, j6). Only minor changes in relationship are present between the groups in this series. Examination of the superimposed outlines in stage 7 shows practically no difference in the form or size of the groups, when normalised on the dimension 7 14 (fig. 12).



FACE

Findings

The findings are presented as described in table X.

Table X FACIAL DATA LOCATION EXPERIMENT 4

Quantity	1 2	1 7	1 14	2 7	2 10	2 11	7 14	11 13	Angles 1 2 8	1 2 9	7 8/1 9	7 8/1 2	1 2 5	Areas 1 2 9 8 7 10 14 1	2 9 14 1 2	7 8 9 14 10 7
Table Longit. Incr.	11	13	14	18	21	22	30	22			47	48	52	58	56	57
Graph Means Log-Log	*	*		*		*										
Graph Means	D4	D6	A6	F4	F5	F6	K6	M6			R5	R6	N6	B6	C4	C5
Graph Individuals	a4					b5		d4			j6	j5	e4	h5	g5	h4

Analysis

Once again, the principal finding is that in the conditions of this experiment, no alteration in the form of the general growth pattern could be observed.

The supremacy in increments of the even rats after stage 6 was maintained in practically all dimensions till stage 9. This may be illustrated by the posterior height of the face (distance 2 10, 2 11, graphs F5, F6) showing a significant inter-group difference at all stages except 2 and 9. The marked difference in the increments between the two groups after stage 7 resulted in a cancelling out of an observable difference in dimensions by stage 9. The same holds true for the anterior face height (1 14, graph A6).

This faster catch-up in the lengths involving rhinion and supradentale conforms with what was found in the previous experiment.

Areas measured in the face showed similar reactions to these in the earlier experiment. The overall facial area (1 2 9 8 7 10 14 1) and the 'oral' area (7 8 9 14 10 7) kept no significant differences in either stage 7 or 9, but the nasal area (2 9 14 1 2) had a significant ($p < 0.05$) difference at stage 7 though none at stage 9.

DENTAL APPARATUS

Findings

The pertinent findings are listed in table XI.

Table XI DENTAL APPARATUS DATA LOCATION EXPERIMENT 4

Quantity	Radius 12 14 15	2 11	10 11	11 13	13 14	14 15	Angles 1 2 11	6 3 11	7 8/14 10	1 2 14	1 2 10	6 3/10 14
Table Longit. Incr.	59	22	31	32	33		53	36	51			
Graph Means Log-Log	*	*										
Graph Means	C6	F6	M5	M6	N4	N5	O5	P6	R4	O6	O4	Q5
Graphs Individuals	d6	b5		d4	d5		e5	e6	i5			i4

Analysis

In this subsection the findings repeat the same general pattern. In the sizes and the relationships, a divergence in the group values occurred which was fully resolved by stage 9, except in the case of the non-growing molar segments and the incisor width (distances 10 11, 13 14, tables 31, 33). As previously pointed out, the molar changes can occur through the fact that the landmarks are located by a combination of non-growing tooth and indeed-growing bone.

The incisor with its continuous growth is different. Comparison of the behaviour of the incisor width and radius of curvature is fruitful in relation to the catch-up phenomenon. It can be seen that the incisor width (13 14, graph N4) is significantly different between the groups at stages 6, 7 and 9. Its increments differ significantly between stages 2 to 6 when the odd rats grow faster, and between stages 7 and 9 when the even ones have the greater increment. Yet the incisor radius (12 14 15, graph C6) now shows a catch-up potential greater than does its width. Its dimension is significantly different only in stages 3 and 6, and the increments show that between stages 6 and 7 the even-numbered rats had achieved a very significant advantage on the rats initially in large litters. The correlation between radius and width was found. At stage 4 it was $r = 0.80$ for the even-numbered rats, and 0.60 for the odd. At stage 6 it was $r = 0.68$ and 0.42, resp. and at stage 7, $r = 0.36$ and 0.11.

SKULL IN TOTO

In Experiment 4 the velocity and acceleration data gave a different picture from Experiment 2. There were highly significant differences between the groups in velocity, with the odd-numbered litters growing faster till stage 7 but at stage 7 and onwards the even-numbered (initially small-litter) rats grew faster.

Exp. 4, Stage	1	2	3	4	wean	6	7	–	9
Post-concep. Days	23	30	37	44	51	58	142	300	500

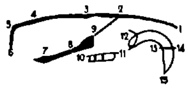


Table XII WHOLE SKULL DATA LOCATION **EXPERIMENT 4**

	Angles													Areas				
Quantity	1 2	1 6	1 7	2 6	2 11	3 9	1 2 5	6 3 1	5 6 1	4 7/ 6 1	7 8/ 6 1	7 8/ 1 2	7 8/ 6 2	2 9 14 1 2	2 3 9 2	1 2 9 8 7 10 14 1	2 3 4 5 6 7 8 9 2	7 8 9 14 10 7
Table Longit. Incr.	11	12	13	17	22	25	52	34	37	44	49	48	50	56	55	58	54	57
Graph Means Log-Log	*	*	*	*	*	*												
Graph Means	D4	D5	D6	E6	F6	H6	N6	P4	T4	W4	S4	R6	S5	C4	B5	B6	B4	C5
Graph Individuals	a4	a5		b4	b5		e4				j6	j5	i6	g5	g6	h5	g4	h4

4 7 And 3 9 were exceptions, because inter-group differences were seen in 4 7 only at stages 2 and 3, and in 3 9 only at stage 3.

Deceleration showed significant inter-group differences in 4 7 at stages 3 and 4, when the greater deceleration was found in the odd-numbered rats. 3 9 At no time showed a significant difference between the groups in this characteristic. The remaining dimensions were found to behave as they did in velocity. It was the odd-numbered rats that decelerated more till stage 7, when as with velocity, the situation reversed.

The effect of this experiment on the whole skull is summarised simply: the change in environment of the litters resulted in an acceleration or deceleration in growth which was of greater magnitude than when the animals were left continuously in the same favourable or unfavourable environment till weaning. Their acceleration in the beginning was counteracted in the animals later in large litters, by the fact that the impact of the sudden change over from good to poor conditions was apparently greater on them than it was on their comparison group in the first experiment, that had been in poor conditions from birth. For whatever reason it may be, they did not achieve the total growth that the above comparison group did. In the other animals in large litters before and in small litters after day 30 (odd-numbered), there was also an effect of their short lasting retardation before day 30 which seems to have caused a limiting of their ability to grow, so as barely to reach even the final growth level of the smaller rats of the first experiment, despite their early acceleration when put in small litters. This is perhaps an unwarranted conclusion. What is however positive is that in this experiment the both groups finished with very much less difference than did the rats of the Experiment 2, although they were smaller in size. So the total effect was the production of a skull of smaller dimensions than in the comparable groups in that experiment, with the form somewhere between those large and small rats.

No material on a similar experiment has come to our knowledge. The work of Widdowson and McCance (1963) resembles it in that an attempt was made to see what effect imposition of underfeeding had on ability to recover, when the time and extent of imposition were varied.

However they did not try to do that before weaning. Their work differed from earlier work on recovery, such as that of Jackson and Stewart (1920), in that they compared later-underfed rats with rats originally reared in large litters. This made it possible for them to see that the effect of later underfeeding was less severe upon the final growth than was the earlier large-litter treatment. Jackson and Stewart had underfed their rats from birth, and formed conclusions similar to those drawn by later workers. They obscured a good deal of their results by the still used but illogical procedure of comparing animals according to weight, regardless of other features. They claimed to show that most of the changes were so that the various parts remained in proportion despite a smaller size. This is not true, and this fault in interpretation may be compared with the mistake of considering the form and shape of a child as just similar to those of a small adult.

A discussion of our findings from this experiment poses some questions that cannot be answered adequately by the information presented. The catch-up in form in this experiment is an impressive achievement, which creates a problem in explaining why the animals of Experiment 2 did not achieve it. One would have expected from previous work that the earlier the change, the greater the impact would be.

Thus the repression from birth continually should have been more severe than repression for just 7 days after birth, or than imposition of restrictions after 7 days of good conditions following birth. Why then were the small rats of Experiment 2 not worse off compared with the both groups from Experiment 4?

A strict comparison of the Experiments 2 and 4 may not be done because among other things they have not been executed simultaneously. Therefore it is not possible to say if indeed it was the experimental conditions in the later study that led to the smaller final size of both groups when compared with the smaller (large-litter) rats of the first experiment.

Should however the circumstances have allowed a proper comparison, then the explanation for the more complete catch-up of the animals in Experiment 4 may have been found in the assumption that while a sudden change from good to poor conditions in the early developmental period after birth affects the growth potential more than a continuous severe condition from birth till weaning, the repression of growth of the animals initially in large litters was an even greater handicap to their growth potential, so as to give the initially small-litter rats the opportunity to overhaul them later on. However at present this is hardly better than a hypothesis.

CONCLUSIONS

The changes in growth rate between the groups have resulted first in a larger size in many dimensions of the even-numbered animals (initially small-litter rats), but this difference disappeared when they were placed in large litters at stage 2. Subsequent to stage 2, the previously smaller rats (initially in large litters) took advantage of the better conditions in a small litter, and out-grew the other group. The increments of the even-numbered rats were the larger till stage 2, after which the increments of the odd-numbered group were significantly larger till after stage 6. In the period between stages 6 and 7 the even-numbered rats generally regained their supremacy in increments. Weaning had occurred between stages 4 and 6.

The consequence of this as found in the cephalometric x-rays, is a similarity of size and form in both experimental groups at the conclusion of the experiment.

In the earlier stages of the investigation, differences were apparent; but the number of dimensions in which there were never any significant differences found was considerable.

From the evidence available it seems that the experimental conditions as used in these experiments have little final effect on the way in which the typical pattern of growth of the many parts of the skull is expressed, since the behaviour of those elements of the skull was in both experiments so far described, closely similar in gross and fine details.

SUMMARY

Extremes of litter size were imposed 2 days after birth but at 30 days post-conception the conditions between the experimental groups were reversed. These rats were then followed till 500 days post-conception.

The effect of the experimental conditions was similar to the previous experiment, but as time passed the inter-group differences became less than had occurred in the previous experiment.

Weight records show that an initial retardation of the large-litter rats in

comparison with the small-litter rats was reversed soon after the change-over of the experimental conditions. Neither group achieved the level of the comparable group in the previous experiment.

No histological differences were found.

The weights of the adrenal glands were also investigated and showed no significant difference in absolute measurements.

The cranial capacity showed significant differences, the rats from large litters after day 30 showing smaller values.

The tooth sizes differed significantly between the groups in M_2 max. and M_1 and M_2 mand.

Study of the cephalometric x-rays made, revealed that the general growth pattern of the skull was similar to the one of the previous experiment, and what appeared to be the primary distinction from the first, regarding inter-group differences, was that the less favoured group was more able to catch-up with the other one than in Experiment 2. It is suggested that the reason for this might be that the more favoured group had been permanently depressed by the initial period in large litters.

As it was realised that the value of the comparison between these two experiments might be increased if a third experiment would produce results conforming with both, a further experiment was set up, where stages 4 and 6 of all experiments would be available for comparison. Further, the advantage of examining the rats after sacrifice at a time much closer to the experimental period was desired for measurement of teeth, adrenals, and cranial capacity.

CHAPTER VII

REPLICATION OF CONTINUOUS IMPOSITION OF LITTER-SIZE EXTREMES: EXPERIMENTS 7 AND 8

In order to examine the possible comparability of the results of Experiments 2 and 4, and to extend the material available, particularly to obtain longitudinal skull data from stage 1 to 2, further series of litters were constructed with some features in common with both the earlier experiments. These experiments were terminated at an earlier age in order to be able to measure the molar teeth directly before attrition was significant, and to determine the cranial capacity and adrenal weight at a time not too far removed from the experimental interference. The rats of Experiment 8 may be further used to examine the growth of the calcified tissues using lead acetate, but this will not be dealt with in this dissertation.

GENERAL INFORMATION

See fig. 7 for a schematic illustration of the design. Both experiments were carried out simultaneously.

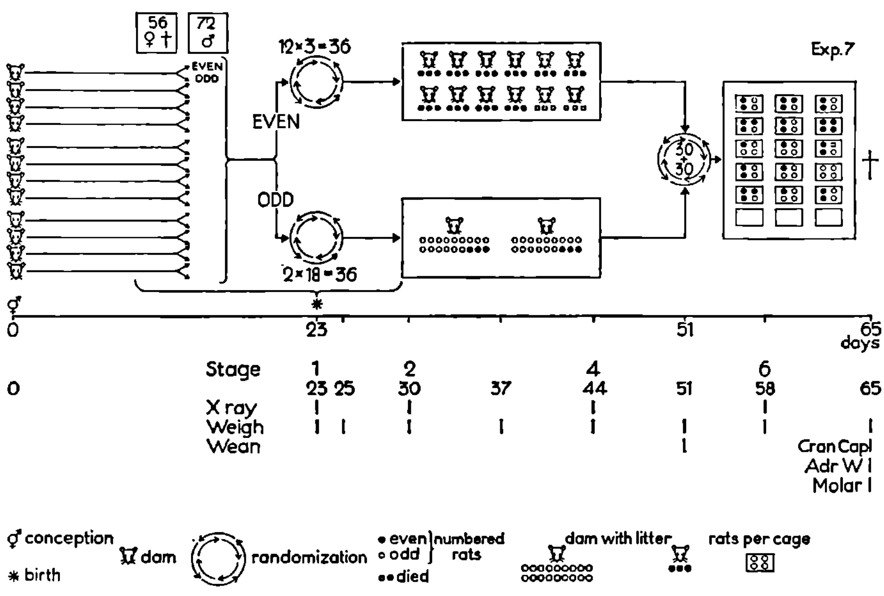


Fig. 7. Schematic illustration of the design of Experiment 7.

Rats conceived on the night of 6/7 March 1967 were born on day 23. Here too the females were discarded, and the males were sexed, numbered and weighed at birth, and redistributed in accordance with a random number table to 12 litters of 3 and 2 litters of 18, odd numbers being in large litters. The first 72 rats were used in Experiment 7; the second 72 in Experiment 8.

The animals of Experiment 7 were used to obtain lateral x-ray pictures on the day of birth and at subsequent occasions, as later will be described. Those of Experiment 8 received injections of lead acetate on days 24, 25, 30, 32, 37, 42, 44, and were sacrificed half on day 44, half on day 51. The dosage of lead acetate was 10 mg per kg body weight intraperitoneally in a solution of 1 mg lead acetate per ml distilled water. Only the growth in weight of these rats is of interest in this work, but the above information is given in case it might be relevant thereto.

The rats of Experiment 7 were weaned at day 51 and caged in groups of 4 according to random numbers as in Experiment 4. On day 65 the rats were sacrificed, the cranial contents aspirated, and the suprarenal glands were removed and kept with the head in 4% formalin. Formalin perfusion was not used, to facilitate suction of the cranial contents.

Compared with the previous experiments, mortality was similar to Experiment 2, being less than in Experiment 4. The figures are to be found in the illustration fig. 7 for Experiment 7 only. Experiment 8 was similar.

In the description of these series the same procedure will be followed as in the two proceeding chapters.

a. SUPERFICIAL OBSERVATIONS

Nothing was seen in these animals to distinguish them from the previous ones.

b. WEIGHT

Method

Weighing was done on days 23, 25, 30, and every 7 days till sacrifice on day 65. In addition, the rats of Experiment 8 were weighed before injection, on days 32 and 42.

Findings

These are given in tables 3 and 4. Fig. 10 gives the weight performance of these rats in graphic form, where they can be compared with the earlier experiments. Note that here the time scale is arithmetic. The standard deviations of two weight records from Experiments 7 and 4 are drawn in as an indication of this characteristic of the mean, but are omitted elsewhere to avoid confusion.

Analysis

The even-numbered rats of both Experiments 7 and 8 show very similar reactions to the experimental conditions. Administration of x-rays on the one hand (in total 300 mr per rat) and lead acetate on the other, made no apparent difference with one exception: the groups of odd-numbered rats did show a difference, in that the lead acetate rats grew significantly better. However the litter sizes were different due to deaths, being respectively 12 and 14 for the lead acetate rats and 15 and 15 for those of Experiment 7 at day 44.

Those of Experiment 8 also fortuitously show a slightly higher mean birth weight, which is known to be associated with higher weaning weight (Parkes 1926). Both these factors may have contributed to the difference.

The experiments taken separately show no significant difference between the groups at birth, nor two days later in Experiment 7. The difference then developed greatly so that at weaning the rats from large litters were approximately 50% of the weight of the rats from small litters in Experiment 7, and 70% in Experiment 8. By day 65 the small rats from large litters in Experiment 7 had caught up to 70% of the weight of the large rats from small litters. As will be realised, Experiment 8 was terminated at day 51, so no further comparison can be made, in this respect.

Discussion

The experimental procedure appears to give quite consistent results. There seems to have been little difference from Experiment 2, despite the different time of the year and other possible disturbing factors. This makes it more likely that the differences seen in Experiment 4 are due to the different form of the experiment. Use of lead acetate injections and the concomitant handling of the animals seemed to cause no more disturbance, perhaps less, than the procedures involved in use of x-rays and ether in the conditions described.

Conclusions

Replication of the experiments, though with minor variations, shows a performance regarding weight gains in the experimental animals, that is similar to that in the original Experiment 2.

Method

The cranial capacity was measured as for Experiment 4, using only the animals of Experiment 7. These were then of 65 days post-conception age (42 days after birth).

Findings

Table 8 gives the means and standard deviations of the measurements, and the correlation between body weight and cranial capacity. The capacities are tested between the groups with the 't' test, as are the correlations after applying Fisher's z transformation.

Analysis

There is a significant difference between the group cranial capacities. They are also significantly smaller than in the older rats of Experiments 2 and 4. The smaller rats have an average capacity of about 20 gm Hg, instead of 28 and 26 as in the corresponding rats in Experiments 2 and 4 respectively, and the larger rats are on average 22 gm Hg in comparison with 29 and 27 for their counterparts. There is a strong positive correlation between cranial capacity and body weight, which correlation is not significantly different in one group from the other.

Discussion

These experiments accord with the results of Diamond et al (1965) mentioned in chapter V. The closeness of the relationship between cranial capacity and body weight was found by them to decrease with age. This is connected with the fact that while brain development is early more complete, body weight continues to change over a very long period. The strong correlation of the Experiment 7 contrasts with the uncertain relationship in the earlier experiments, with rats of a much more advanced age when measured. The similarity of the correlations exhibited by both experimental groups is also of significance, in that it indicates that the experimental conditions themselves did not create such a difference in the two groups that their cranial capacity to body weight relationships were made to differ noticeably. From this it may be concluded that the proportional relationship between cranial capacity and body weight is rather strictly regulated.

A cross-sectional comparison between the series of Experiments 2 and 7 for an evaluation of the increase in mean cranial capacity between day 65 and day 500 gives a 39% increase in the odd-numbered rats and a 35% increase in the even-numbered ones.

This finding indicates a catch-up taking place in cranial capacity after day 65, in that the growth rate decelerated less in the odd-numbered rats. Returning to the findings in Experiments 2 and 4, referred to on page 67, we saw that although there were different degrees of catch-up in body weight between the series, the catch-up in cranial volume was similar in both. We now see that in this experiment the cranial capacity exhibits an inter-group difference not greatly differing from that in Experiment 2 at 500 days, while the body weight difference is much greater. This indicates that the catch-up in cranial capacity is limited, and no doubt that is due primarily to the fact that cranial growth is earlier near completion than weight gain. This will be seen again when considering related longitudinal measurements of area on the cephalometric x-rays. The assumption of comparability of the material from the both experiments is confirmed by those data.

Conclusions

The experimental conditions led to a significant difference in the cranial capacity of the two groups by day 65. The cranial capacity was strongly correlated ($r = 0.7$, $n = 30$) with the body weight at sacrifice in both groups.

d. ADRENAL GLAND WEIGHT

Method

The method was identical with that of Experiment 4.

Findings

Table 8 gives mean weights and standard deviations of both groups. Correlations between body weight and adrenal gland weight are given. Relative weight of adrenal glands expressed as mg/100 gm body weight is given in the same table.

Analysis

There was a significant difference in the weights of the adrenals of the experimental groups. Both groups showed a significant correlation between body weight and adrenal weight. The correlation is not significantly different for the two groups. Relative adrenal gland weight was significantly larger for the odd-numbered rats, whereas their absolute gland weight was smaller.

Discussion

At this time shortly after the experimental conditions were removed, a more obvious relationship between those conditions and the adrenal glands is revealed than in the previous Experiments 2 and 4. The relative weight of the adrenals is greater in the stressed group. The absolute gland weight is significantly and positively related to the body weight in both groups. This corresponds with the results of the other workers previously mentioned.

Compared with the previous experiments, the adrenals are considerably smaller. The weight of the adrenals of the odd-numbered rats of Experiment 2 at 500 days is about twice the weight of the adrenals of the equivalent rats in Experiment 7 at day 65. With the even-numbered rats this increase is about 72 %. This indicates a considerable catch-up in adrenal growth in the odd-numbered rats from the aspect of absolute size. However, the relative weight of the glands falls considerably with age.

Conclusions

The experimental conditions promote in the animals in large litters observed at day 65, a higher ratio of the weight of the adrenal glands to the body weight (i.e. relative adrenal weight) than in animals reared in small litters. Nonetheless the absolute weights of the glands are still significantly smaller in the former animals than in their counterparts. The results are much more definite in this experiment than in the previous ones, and justify the assumption that adrenal glands are more active in stress conditions, accepting that weight measurements are usable estimates of this activity.

e. HISTOLOGY

Method

This was the same as in the last experiment.

Findings

The lipoid content of the adrenals was seen to differ. A reduction of lipoid, with clumping, was apparent in the large-litter group (odd-numbered) when appropriately handled sections were examined under polarised light. In other respects no difference was discernible between the groups.

Discussion

The lipoid content of the adrenals may be affected by several factors, including sexual maturation and stress. Since these animals are at about the age of puberty, and are probably recovering from the stress of large litter-size in the odd-numbered group, both factors may be playing a part. Whichever it may be, a difference is to be seen. If sexual maturation is the cause of the difference, then it is evidence that the experimental difference has created a difference in the time of maturation of the groups so that only one of them shows the lipoid disturbance.

Conclusions

In contrast to the investigation of the adrenal glands of older animals, where the picture was normal in both groups, in this series an experimental difference was found in the distribution of lipoid in those glands. It was depleted in the large-litter group.

Other histological differences were not found.

f. MOLAR SIZE

Direct measurements of the mandibular molars were performed to check the results of the x-ray measurements and to extend the records to bucco-lingual dimensions.

Method

After sacrifice the left mandibles were removed and cleaned of soft tissues by scraping. The section of the bone bearing the molars was separated from the rest with a diamond disc, leaving the molars intact. These blocks were then mounted on bases of self-polymerising polyester resin so that 5 blocks were arranged in line on one base, with the occlusal surfaces touching the same plane parallel to the bottom of the embedding plastic. The shrinkage of the polyester in processing had no relevant effect on the blocks of tissue. The crowns of the teeth were left exposed and the inclination of the teeth bucco-lingually was adjusted in as closely similar a direction as could be obtained. The blocks

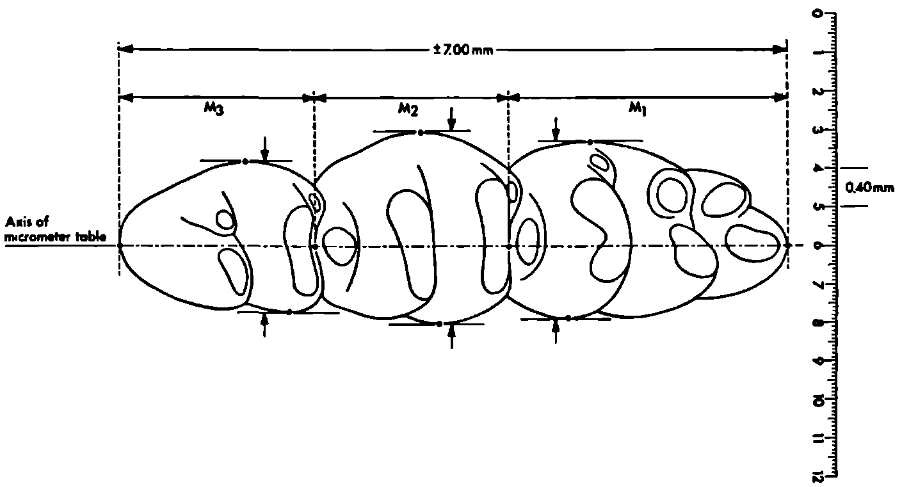


Fig. 8. Arrangement of measurements taken directly from mandibular molars.

retained the identity of the rat from which they came, and were placed on the bases in an order derived from random number tables to reduce bias in measuring errors.

The prepared blocks on their bases were kept in 4% formalin and were blown dry with compressed air before measuring. Measuring was performed by placing the blocks on the Durimet microscope table with one axis of the table in line with the common mesio-distal axis of the molars of each block, as illustrated in fig. 8. At right angles to this axis an ocular micrometer was installed in the dissecting microscope mounted above the Durimet table described in Chapter IV (page 26). A magnification of 25 X was used, when one division of the ocular micrometer was the equivalent, measured by the micrometer-driven cross-table, of 393×10^{-4} mm. This is simplified to 400×10^{-4} in the figure 8 where the distances measured are illustrated. The measurements were done once from mesial to distal, then repeated from distal to mesial. The ocular micrometer was used for bucco-lingual measurements only, and at the given magnification the width of one molar occupied about $^1/_4$ of the width of the field.

Individual and total mesio-distal lengths were measured.

The standard deviation of single determinations was estimated from the formula $s_i = \sqrt{\frac{\Sigma d^2}{2n}}$ (Dahlberg 1940). It was found to be negligible in the case of bucco-lingual measurements, and for mesio-distal measurements was $\pm 1.0 \times 10^{-2}$ mm, when n was 10.

Findings

The findings are given in the usual form in table 9. Correlations between mesio-distal and bucco-lingual dimensions have been calculated and the probability that 'r' might be zero has been indicated.

Analysis

There is a significant difference between the experimental groups with respect to mesio-distal lengths of M_1, M_3, and the sum of all the molars. Even-numbered rats show greater molar size. The same applies to molar width, now for the M_2 and M_3. The value for 't' for the M_3 difference is considerably greater than the other values.

The values for 'r' show little correlation between length and width for M_2 and M_3 but a significant correlation for M_1 in the odd-numbered rats. In the even-numbered rats the correlation is significant in the case of M_1 and M_3, and also the value for M_2 is considerable higher than in the other group. This indicates a relatively constant form for M_1 in both groups, but less constancy of form in the other molars in the odd-numbered group.

Discussion

These findings correspond with other work mentioned before, and support the findings of the x-ray measurements despite the various differences in the material and techniques.

The lack of a significant difference in M_2 cannot be accounted for from the present data. It should be born in mind however that in no case in the data has there been a mean dimension in the odd-numbered group that was larger than that in the even-numbered group. Statistical significance in such circumstances can be regarded as a useful milepost, but not to have reached it does not mean one is on the wrong road.

The information given by the correlation coefficients seems to indicate a greater constancy of form in teeth that grew under optimal conditions in this experiment. It is not possible to discern why that should be so, and the comparison of correlation coefficients of low value is of dubious significance. Others have found a closer correlation ($r = 0.781$ for M_1) between bucco-lingual and mesio-distal width using 'normal' rats, and different landmarks and technique (Johannessen 1961).

Conclusion

An experimental effect has been the production of a difference in mandibular molar sizes between the groups, so that rats reared in large litters had smaller teeth than the other rats.

Table XIII — CRANIAL BASE DATA LOCATION — EXPERIMENT 7

									Angles								
Quantity	2 7	2 8	2 9	3 9	4 7	4 8	7 8	7 9	8 9	7 8 9	8 9 2	4 7 8	7 8/1 9	7 8/6 1	7 8/6 2	7 8/1 2	6 5/7 8	5 6 3
Table Longit. Incr.	18	19	20	25	27		29			46	45	43	47	49	50	48	41	38

Table XIV — CRANIAL VAULT DATA LOCATION — EXPERIMENT 7

									Angles							Areas	
Quantity	2 3	2 5	2 6	3 4	3 7	3 9	4 5	4 7	5 7	1 2 5	5 4 3	5 6 3	6 5 4	7 8/1 2	7 8/6 2	6 3/4 7	2 3 9 2	2 3 4 5 6 7 8 9 2
Table Longit. Incr.	15	16	17	23	24	25	26	27	28	52	40	58	39	48	50	35	55	54

Table XV — FACIAL DATA LOCATION — EXPERIMENT 7

										Angles			Areas		
Quantity	1 2	1 7	1 14	2 7	2 10	2 11	7 14	11 13	1 2 8	1 2 9	7 8/1 9	7 8/1 2	1 2 5	1 2 9 8 7 10 14 1	2 9 14 2	7 8 9 14 10 7
Table Longit. Incr.	11	13	14	18	21	22	30	22			47	48	52	58	56	57

Table XVI — DENTAL APPARATUS DATA LOCATION — EXPERIMENT 7

	Radius						Angles					
Quantity	12 14 15	2 11	10 11	11 13	13 14	14 15	1 2 11	6 3 11	7 8/14 10	1 2 14	1 2 10	6 3/10 14
Table Longit. Incr.	59	22	31	32	33		53	36	51			

Table XVII — WHOLE SKULL DATA LOCATION — EXPERIMENT 7

							Angles							Areas				
Quantity	1 2	1 6	1 7	2 6	2 11	3 9	1 2 5	6 3 1	5 6 1	4 7/6 1	7 8/6 1	7 8/1 2	7 8/6 2	2 9 14 12	2 3 9 2	1 2 9 8 7 10 14 1	2 3 4 5 6 7 8 9 2	7 8 9 14 10 7
Table Longit. Incr.	11	12	13	17	22	25	52	34	37	44	49	48	50	56	55	58	54	57

Method

The animals were used for x-ray cephalometry on days 23, 30, 44, and 58. Lateral pictures alone, were taken.

The information gathered from the x-rays will be treated here as a whole; a division in subsections is not now considered meaningful.

Findings

The presention of the data is limited to the tables of increments, and means at stage 6, which appear in tables 11 to 59. With only 4 registrations, little of value was expected of graphs, as this meant that most dimensions would have only 3 points and several only 2. See tables XIII to XVII.

Analysis

It will be seen that the mean outlines (figs. 11 and 12) show the same characteristics as in earlier experiments, though size comparisons will show that mean values of the rats are often larger (H_0: $p < 0.05$) in stage 4 than those of Experiments 2 and 4. Stages 4 and 6 of all three series can be compared. In stage 6 the rats of Experiment 2 are larger (H_0: $p < 0.05$) when a significant difference occurs and their angular differences from Experiment 7 correspond with the normal trend. For example, the anterior cranial base angle is flatter in the larger Experiment 2 animals (H_0: $p < 0.001$) and the posterior angle is smaller (H_0: $p < 0.05$). The Experiments 4 animals are smaller again than those of Experiment 7 at stage 6 (H_0: $p < 0.01$), and give the impression that the form of the corresponding groups (large-litter rats compared with large-litter rats, for example) is similar though it is apparent from the tables that this is a rough approximation.

It is interesting to observe the real longitudinal increments of stages 1 to 2 at last! There is no reason to assume that there is a marked difference in the growth behaviour of the first week of the three experiments.

The mean values in the last comparable stage, stage 6, are quite similar between Experiments 2 and 7. 't' tests confirmed this impression. Significant differences only occurred in the more rapidly growing regions where probably the small differences between the experiments could be expected to reveal themselves. Tests showed the Experiment 2 rats were larger than those of Experiment 4. Stage 6 in Experiment 2 occurs 4 days later than in Experiment 4. This may help explain such systematic size difference, though one hardly expects consistently to measure 4 days' growth at that age!

Study of the increments shows a peculiarity in the first week, where in several dimensions the odd-numbered (large-litter) rats have the advantage. This is also seen in the weight records of this experiment (tables 3 and 4).

The conditions in Experiment 4 over the early period were fairly similar to those in this experiment, so similar results might be expected. The occurrence of significant differences at stage 2 does not conform in both experiments, nor do the mean outlines correspond very well. The difference in Experiment 4 of imposing conditions at day 25 may be responsible for this.

Tests between the series, of representative quantities at stage 2, indicated no significant difference between the inter-group differences of both series.

DISCUSSION

It is difficult to discuss the most notable observation from this series, the greater increments in some linear measurements seen in large-litter rats in the first week after birth. It is not at all unlikely that this would be disguised if the increment were taken over a longer period. It would be interesting to make even more frequent observations to see just when the superiority of increments was gained. There is no doubt that in general the growth of the large-litter rats was suppressed till weaning, and that the increments measured genuinely longitudinally showed their increments to be less than the small-litter rats. It may be that despite the ready susceptibility of the weight to depression by the conditions, the skeletal growth had sufficient impetus from the intra-uterine environment to maintain growth at a high rate for a short time. After two weeks, when the first increment was measured in the other series, the depression may have occurred to such an extent that the first week's gains were no more to be recognised in comparison with the well nourished group. Only further work would elucidate this.

CONCLUSIONS

The replication of the experiments produced, as far as can be seen, a reaction in the animals that is satisfactorily closely similar to that in the original Experiment 2.

The incremental data in the initial week show significant inter-group differences in general, and give another indication of the direction of the difference than that expected. It appears that the large-litter rats showed more increment in the first post-natal week than the small-litter rats, in the majority of dimensions. It cannot however be directly compared with that period of any other experiment since no x-rays were taken in the latter at birth.

SUMMARY

Replication of Experiment 2, with cephalometric x-rays taken at birth, and with sacrifice of the animals at the post-conception age of 65 days, showed that similar results could be obtained.

In this case four groups of 36 rats were used for weight records, two groups of which received lead acetate injections. The large-litter group of these two showed more favourable reaction to the experimental conditions than did those not receiving lead acetate but ether and cephalometric x-rays instead, with their associated procedures.

At the age of sacrifice, histological examination did show a difference in the adrenal glands. The large-litter rats showed depletion of the lipoids which is a possible sign of stress reaction. The weight of the adrenals showed a strong positive correlation with body weight and exhibited significant inter-group differences in weight. Relative adrenal weight was significantly different, the small-litter rats having smaller relative weights.

Mandibular molar size was measured directly and showed significantly larger molar size in small-litter groups, with respect to mesio-distal length of M_1, M_3 and the total molar segment. Bucco-lingual width of M_2 and M_3 showed the same difference.

From the correlation between bucco-lingual and mesio-distal molar widths, it appears that M_1 is more constant in form than M_2 and M_3.

The cephalometric investigation showed an unexpected fact, in that the increments in the first 7 days were in several instances in favour of the large-litter rats. The rest was in agreement with the previous findings.

CHAPTER VIII

GENERAL DISCUSSION

Many facets of our study have already been mentioned and are discussed in the pertinent parts of this thesis.

The findings of other investigations have been brought in mainly in the chapters where the experiments were presented and they have been incorporated in the discussion of our own observations. Due to this the general discussion can be limited to the aspects involving co-ordination of observations made in different chapters and to some general remarks.

Rearing of male rats in extremes of litter-size resulted in a marked difference between the two groups, noticeable a few days after imposition. The difference gradually reduced after the elimination of the extreme conditions upon weaning, but did not disappear completely. Even at day 500 the weights still showed significant differences as did most of the other registrations. The superficial observations, including hair-formation, opening of eyes, and formation of external ears as well as the histological evaluation of certain tissues, with exception of the adrenal glands, did not indicate that a difference in physiological conditions was present between experimental groups.

From our experiments it became clear that the impact of early changes in environmental conditions is considerable and can have a permanent effect on size and shape. This has for example been shown for the body and tail length, the cranial capacity and the weight of the adrenal glands. The alterations in size, but particularly those of shape, clearly show up in the craniofacial skeleton as can be seen from the illustrations of the mean rat outlines (figs. 11 and 12). From our findings we are able to say that a relatively slight degree of under-nutrition in early life, such as is present with litter-size in rats, will affect the growth of the head in such a way that the cranial vault is slower to flatten, the face is slower in growing upwards and forwards, and the cranial base is slower to straighten anteriorly and to bend up posteriorly.

It has been apparent that the experimental conditions made no discernible difference to the general pattern of growth of particular parts of the skull; the differences were rather in rate of growth. From examining the way in which the parts behaved, we have seen that the cranial height reacted in quite a different way from the cranial length; and the latter, the snout, and cranial base seemed to be related closely in behaviour.

The development of the teeth and their final dimensions definitely depend on environmental conditons during their period of formation. The integration of data presented by Schour and Massler (1949) and Paynter and Hunt (1964) on tooth formation in rats, and our findings, makes it clear that the teeth that showed the inter-group differences were the ones passing through a rapid stage of formation during the period of intervention. This explains also why the observations on the size of molars in Experiment 2 and 4 were different. It may be remarked here that the reduction of inter-group differences as time went on, in general present in all other registrations, is not found for the molars. There the differences have a permanent character concomitant with the stability in size of molar crowns once calcified.

Observations on the adrenal glands proved to be more fruitful when the animals were sacrificed soon after release from the experimental conditions. Those from animals sacrificed at 500 days had shown only a weight difference resembling the other inter-group differences in size. The rats sacrificed on day 65 showed not only a weight difference in the adrenals but also a histological inter-group difference in those glands. From the latter it was evident that the large-litter rats had reacted with a change in the activity of their adrenal cortex. We could not show if it was stress, sexual maturation or some other factor that was responsible for the hypertrophy and lipoid depletion found, but the relationship with the difference in environment was clear.

The determination of the cranial capacity in the different experiments led to a better insight into the relationship between the size of the skull and the cranial capacity. The cranial capacity showed a distinct growth picture as it reached its final size earlier than the skull as a whole. Its possibility for catch-up is consequently reduced, since the remaining growth is relatively limited. This explains how it was that the close correlation between cranial capacity and body weight at the post-conception age of 65 days was not to be found at day 500. Early brain growth (Sugita 1918, Eayrs and Horn 1955) is related to early cranial growth (Weidenreich 1941, Moss 1954) though a causal relationship has not been proven. Prahl (1968) indicates that in the calvaria of the rat between 27 and 34 days post-conception the sutural growth, and not that of the brain, is the primary agent in increase in size of the cranium. This statement is of particular interest when one considers the slower elimination of doming of the cranial vaults in the large-litter rats. A brain growth of a relatively greater magnitude than that of the calvaria would tend to result in a cranial content of a larger volume than the capacity of the cranium of normal form could accomodate. A more spherical shape would enable the same enveloping tissue to encompass a greater volume. This then offers a possible explanation of the slower straightening of the vault in the large-litter rats, i.e. that it results from the differences in growth rates and catch-up already mentioned.

The fact that we have been able to show that definite, differing growth patterns are present in the components of the cranial vault may be considered as a support for the proposition that the brain is not the leading factor in the development of the cranium.

Another possibility that does not conflict with the previous one may be offered in explaining the shape differences of the skull. It has been contended (Park and Richter 1953, Acheson 1958, Park 1964) that chondrogenesis and osteogenesis are separable, and dissociation has been produced in severe environments. As the growth of the cranial base and face may depend more on chondrogenesis (which is said to be earlier depressed than osteogenesis) than would that of the cranial vault, it may be that the doming and the slow upward and outward facial growth in the large-litter rats can be attributed to a relatively greater reduction in growth of the cranial base and the face than in that of the vault. Experiments seeking concrete information on the importance of the cranial base as an active growth agent in the skull are currently going on in the laboratory where this work was carried out.

At present it is felt to be unjustified to make a more definite statement regarding the manner in which the skull shape differences come about. Consequently we shall limit ourselves to the following facts:

Environmental conditions have a considerable effect on the process of growth and catch-up, in male Wistar rats. This causes not only changes in size but also in shape, as can clearly be observed in the craniofacial skeleton.

There is a positive relationship between small size and large litter-size in male Wistar rats, associated also with rounder calvaria, less elevated faces, straighter posterior cranial bases and more concave anterior cranial bases.

Environmental influences on the general growth pattern are insignificant; their influence is primarily on intensity of growth.

Different degrees of catch-up in growth can be expected according to the time and extent of depression of growth.

Tooth size is definitely susceptible to environmental influence.

Growth in the rat skull persists in most dimensions at least till 500 days.

Results of animal experiments must be evaluated with the possibility in mind that relatively small environmental differences can produce changes in size and shape. Experimental morphology is especially vulnerable to this.

CHAPTER IX

SUMMARY

The purpose of this study was to investigate the influence of environmental factors on size and shape changes in growing individuals. Several disciplines are interested in this field. The orthodontist's attention goes primarily to the growth of the head and in particular to that of the face. It is apparent that environmental influences ranging from the natural soft tissue integument, an infantile thumb, the regular playing of the clarinet, to any of the many orthodontic appliances, will to a certain extent, and at least temporarily, mould the hard tissues of the head and face to a pattern other than that in which they originally grew.

In this work the influence of a specific environmental factor on the growth and development of the rat, including a detailed analysis of the growth pattern of the cranio-facial skeleton, has been investigated. It was felt that this could form a contribution towards a better understanding of the interaction between genetic and environmental factors on growth. In the first chapter we have described how the genetic composition of an organism, though determined at conception, is dependent on the environment for its fulfilment.

The interaction between genetic and environmental factors was discussed, and the importance of the more or less constantly changing aspects in this was indicated. Several disturbing mechanisms were mentioned.

The influence of nutrition was considered more specifically. However, it was also remarked that the mechanisms by which growth is regulated are admitted to be largely unknown.

The second chapter has been used to clarify the concept of a growth pattern, and to describe how we have attempted to provide a simple expression of this for the animals we studied. Emphasis was placed on the fact that growth is of necessity dynamic, which introduces specific problems in its description.

With this background we proceeded to detail the experimental work itself in Chapter III. Here were considered the possible environmental influences that could be used: alteration of the plane of nutrition, acute infection or acute starvation, alteration of physical environment, administration of drugs, hormones and other media, and birth-size regulation. These were described and evaluated with reference to previous work. Factors of importance in the manner of application of the conditions were then dealt with. Effects of the environment depend mainly on severity of treatment, time and duration of application, type of animal and the sex of the subject. The importance of these aspects was given relevance to the choice made of the approach of imposing extremes of litter-size on male Wistar rats at birth, and maintaining the difference till weaning. Litter-size manipulation was described in some detail, and explanations for the phenomena observed in its use were given. The plane of nutrition is strongly related to size in young growing animals. The factors affecting this in the litter were discussed.

In Chapter IV the animals and their rearing have been described. Randomisation was used to eliminate the genetic effects from the samples, which were paired. The specific environmental difference was made to provide the only experimental variable. Half of the animals were reared from birth to weaning in litters of three rats per dam, and the other half were reared in litters of 15 to 18 rats per dam. After weaning both groups were caged in groups of four, and fed ad libitum. Weaning was done 51 days post-conception (28 days post-natal). Longitudinal records were taken of various features: in particular body weight, tail and body length, and standardized cephalometric x-ray pictures of the rats on several occasions. After sacrifice, cranial capacity, adrenal weight, and, in the last experiment, mandibular molar size, were measured and several tissues histologically evaluated.

A detailed account of the cephalometric technique was also given in Chapter IV. The importance of correct treatment of longitudinal data was pursued. The volume of the data from the cephalometric records, in total over 20,000 measurements, necessitated handling by computer (I.B.M. 360/50). The points used are illustrated in plate IV and its legend. The output has provided selected tables of longitudinal incremental data, with key sample means. Mixed longitudinal data have been reproduced mainly in the form of graphs to provide ready access to, and a visual impression of, pattern. Graphs of individual growth data were also provided. In addition, computer-constructed outlines of rat skulls compiled from mean values, have been superimposed in various ways.

In Chapter V the first experiment, called Experiment 2, was dealt with, in which 64 rats were subjected to continuous extremes of litter-size from birth to day 51 post-conception (figs. 1 and 2). Already after 2 days it became clear that the experimental difference resulted in a significant difference in the rats. The weight gain of the small-litter rats became greater than that of the others (see table 1). The tail-body length ratio became lower for those rats (table 7), and growth in almost all dimensions was greater (tables 11 to 59). However, after weaning, the rats which had been in large litters decelerated less in their rate of growth and, particularly in their skull dimensions, began to approach their counterparts.

The differences in growth increments in skull dimension became significantly in favour of the large-litter rats (tables 11 to 59), but when they were sacrificed 500 days after conception, the large-litter rats had not yet made up for the initial loss of growth.

Cranial capacity and adrenal weight measured after sacrifice showed significantly smaller size in the large-litter rats (table 8).

No histological differences were discernible between the groups in the sections of adrenal glands and skulls studied.

Measurement of the x-rays of maxillary molars revealed significantly smaller lengths of the total molar segments, and of the mesio-distal widths of the second and third molars (table 9). This was related to data from the literature on the tooth development of the rat.

The cephalometric study of this experiment revealed as is shown primarily in the tables and graphs and mean outlines (tables 11 to 59, graphs A1 to W1 and figs. 11 and 12), that the typical pattern of growth was practically the same in both groups. Graphs a1 to j1 give individual patterns.

In the analysis of the x-rays taken at days 36, 43, 62, 142, 300, and 500, the skull was initially studied in 4 regions: cranial vault, cranial base, face, and dental apparatus.

The calvaria were seen to develop most in the early weeks of life, and this was particularly noticeable in the height dimensions. The form of the cranial vault was initially bulbous but straightened with age. Comparison between the two groups showed a corresponding general pattern in timing, with difference in intensity; almost all linear dimensions registered were found to be significantly greater in the small-litter rats.

Large-litter rats had more bulbous crania, though the differences decreased with time. They were slower (figs. 11 and 12) to straighten out their cranial vaults, and in the growth upwards and outwards of their faces.

The cranial base grew evenly and gradually over the period studied. This corresponded with the vault length growth. The angle between sphenoid and presphenoid decreased with age, and that between presphenoid and the cribriform plate increased after day 43. The angular relationship of the face to the cranial base showed an early rapid change.

Intergroup differences generally showed larger dimensions for small-litter rats. There was a more or less complete catch-up in the cranial base elements anterior to the sphenoid bone, being the ones most closely related to the facial structures. The angle at the spheno-ethmoid suture showed no noticeable difference at any stage although the angle at the spheno-presphenoid synchondrosis was significantly smaller in the small-litter rats from day 36 onwards.

The face was found to grow initially much more slowly than the cranial measurements, but soon the relative increments became higher than in the cranium. Then angular changes with growth in the face reflected a growth forwards and upwards relative to the cranial base.

Inter-group differences in the face were considerable. The small-litter rats had larger height and length dimensions. The catch-up in the height anteriorly seemed to be more or less complete, in contrast to that in the posterior face height. The catch-up in the face occurred later than in the cranial structures.

The dental region showed the differences in molar size mentioned before. The incisor width and radius of curvature grew in the same general pattern as the majority of dimensions: evenly over the whole period studied. The orientation of the dental plane changed with age in relation to the body of the sphenoid.

Comparison of these features between the groups showed that the incisors were also smaller in the large-litter rats, in both width and curvature. The large-litter rats had a less vigorous increase in posterior face height as indicated by the dental plane which differed between the groups in all stages. The angle between this plane and the sphenoid was larger in the large-litter animals.

Consideration of the skull in toto showed how the ventral components of the skull grew more than the dorsal. Their part in elevating the face was accomplished by day 43. The remainder of the straightening of the top of the head came from re-orientation of the vault components and changes in the angle at inion.

Intergroup differences in the skull in toto gave the impression that the large-litter rats showed a relatively greater difference in growth of the dorsal and ventral regions, so that less straightening of the vault occurred, and the face elevated more slowly.

The mandible was studied on purely longitudinal material recorded at days 62 and 142. The findings showed a close correlation with the growth of the face. However, despite considerable inter-group differences in size, there was barely any in shape. A marked catch-up occurred in size over the period studied (fig. 4).

Study of velocity and acceleration data from fitted curves indicated few significant differences between groups, though those that there were indicated that after weaning the large-litter rats grew faster and decelerated least.

To see what effect an earlier change-over in environmental conditions might secure, another experimental series was set up using 72 rats (Experiment 4, figs. 5 and 6 Chapter VI). These were treated similarly to those in Experiment 2 (the first one) except that now the conditions of litter-size extremes were not imposed till day 25. Then at day 30 the conditions were reversed by splitting the large litters into small ones, and amalgamating the small litters into large ones.

Thus the animals originally in small litters found themselves after day 30 to be in the unfavourable circumstances of a large litter, and vice versa. Apart from tail/body ratio, the same records were taken as in the previous experiment, but at slightly different times.

The findings showed that the reversal of the conditions was followed promptly by a reversal in the differences between the groups in growth. Weight records showed that the weights of the corresponding rats of this and the previous experiment showed a significant difference, those of this experiment being lower. The effect of the experimental conditions was similar to the previous experiment, but as time passed the inter-group differences became less than had occurred in Experiment 2. The timing of the experimental differences was reflected in the growth of the molars, since the sizes showed now larger M_2 in the maxilla and M_1 and M_2 in the mandible, of rats then in large-litters but originally in small ones.

Study of the cephalometric data revealed the same general growth pattern of the skull as in the previous experiment.

With respect to the inter-group differences, it became clear that the less favoured group was more able to catch up with the other one than was the case in Experiment 2.

It was not till between days 58 and 142 that the increments of the originally small-litter rats became significantly greater than those of the other group. Velocity and acceleration data showed how the originally small-litter rats grew faster and decelerated less after day 142.

In Chapter VII two experiments (Experiments 7 and 8, fig. 7) were described, both of the same design as Experiment 2. The first, involving 72 rats was set up to gain more information at earlier stages, and to confirm some observations. Of the second series also of 72 rats, only the weight records were used. Good similarity was found with the corresponding details of Experiment 2. Sacrifice of the animals at day 65 provided the desired additional information on the effect on the adrenals and the cranial capacity. Besides obvious differences in weight – and now in contrast to the previous results at 500 days – differences in the histological appearance were detected in the adrenals, providing support for the hypothesis that differences in environmental stress had induced differences in the activity of those glands. At this age too, the cranial capacity was more closely correlated with body weight than in the other experiments at day 500.

Molars measured directly in the mandible showed significant inter-group differences in M_1 and M_3 and in the total molar segment. Bucco-lingual width of M_2 and M_3 was similarly larger in the small-litter rats. Cephalometric findings confirmed previous results. However, increments in the first stage interval showed an advantage for the larger-litter rats. An explanation for this cannot be given, especially since there was no comparable period studied in the other series.

In the general discussion in Chapter VIII, some findings provided in the earlier chapters have been co-ordinated and some general remarks made. In particular, a possible explanation for the shape changes in the skull was sought in the differential effects of environment on brain and skull growth, and their catch-up. Another possibility not contradicting the previous one in explaining the shape differences of the skulls, and faces, was based on a difference in susceptibility of chondrogenesis and osteogenesis to stress.

As the most essential conclusions, the following were given:

Environmental conditions have a considerable effect on the process of growth and catch-up, in male Wistar rats. This causes not only changes in size but also in shape, as can clearly be observed in the craniofacial skeleton.

There is a positive relationship between small size and large litter-size in male Wistar rats, associated also with rounder calvaria, less elevated faces, straighter posterior cranial bases and more concave anterior cranial bases.

Environmental influences on the general growth pattern are insignificant; their influence is primarily on intensity of growth.

Different degrees of catch-up in growth can be expected according to the time and extent of depression of growth.

Tooth size is definitely susceptible to environmental influence.

Growth in the rat skull persists in most dimensions at least till 500 days.

Results of animal experiments must be evaluated with the possibility in mind that relatively small environmental differences can produce changes in size and shape. Experimental morphology is especially vulnerable to this.

ACHESON, R. M. and HEWITT, D. (1954): *Oxford Child Health Survey. Stature and skeletal maturation in the pre-school child.* Brit. J. Prev. Soc. Med. **8**: 59–65.

ACHESON, R. M. and MACINTYRE, M. N. (1958): *The effects of acute infection and acute starvation on skeletal development.* Brit. J. Exp. Path. **39**: 37–45.

ACHESON, R. M., MACINTYRE, M. N. and OLDHAM, E. (1959): *Techniques in longitudinal studies of the skeletal development of the rat.* Brit. J. Nutr. **13**: 283–292.

ASLING, C. W. and FRANK, H. R. (1963): *Roentgen cephalometric studies on skull development in rats. I. Normal and hypophysectomized females.* Amer. J. Phys. Anthrp. **21**: 527–544.

BECKS, H., COLLINS, D. A., ASLING, C. W., SIMPSON, M., LI, C. H. and EVANS, H. M. (1948): *Gigantism produced in normal rats by injection of the pituitary growth hormone. V. Skeletal changes: skull and dentition.* Growth **12**: 58.

BJORK, A. and SOLOW, B. (1962): *Measurement on radiographs.* J. D. Res. **41**: 672–683.

BODEGOM, J. C. (1969): *Experiments on tooth eruption in the miniature pig. M.D. Thesis,* University of Nijmegen.

BONNER, J. T. (1952): *Morphogenesis.* Princeton University Press. N. J.

BRODIE, A. G. (1941a): *On the growth pattern of the human head from the third month to the eighth year of life.* Amer. J. Anat. **68**: 209–262.

BRODIE, A. G. (1941b): *Behaviour of normal and abnormal facial growth patterns.* Amer. J. Orthodont. **27**: 633–647.

BRODY, S. (1945): *Bioenergetics and Growth.* Reinhold N.Y.

BURCH, P. R. J. and BURWELL, R. G. (1965): *Self and not-self. A clonal induction approach to immunology.* Quart. Rev. of Biol. **40**: 252.

CARMON, J. L., GOLLEY, F. B. and WILLIAMS, R. G. (1963): *An analysis of the growth and variability in Peromyscus Polionotus.* Growth **27**: 247–254.

CASTLE, W. E. (1922): *Genetic studies of rabbits and rats.* Carnegie Inst. Washington. Publ. no. 320.

CLOWES, R. (1967): *The structure of life* (p. 291). Penguin Books London.

DAHLBERG, G. (1940): *Statistical Methods,* Allen and Unwin, London,

DIAMOND, M. C., ROSENZWEIG, M. R. and KRECH, D. (1965): *Relationships between body weight and skull development in rats raised in enriched and impoverished conditions.* J. Exp. Zoology **160**: 29–36.

DICKERSON, J. W. T. and MCCANCE, R. A. (1961): *Severe undernutrition in young and adult animals. 8. The dimensions and chemistry of the long bones.* Brit. J. Nutr. **15**: 567–576.

DICKERSON, J. W. T. and WIDDOWSON, E. M. (1960): *Some affects of accelerating growth: Skeletal development.* Proc. Roy. Soc. B. **152**: 207–217.

DIKSHIT, P. K. and TASKAR, A. D. (1956): *Role of mammary glands on weight of suckling rats.* Proc. Soc. Exp. Biol. Med. **93**: 235–238.

DRIEZEN, S., SNODGRASS, R. M., WEBB-PEPLOE, H. and SPIES, T. D. (1958): *The retarding effect of protracted undernutrition on the appearance of the post natal ossification centers in the hand and wrist.* Hum. Biol. **30**: 253–264.

DUBRUL, E. L. and LASKIN, D. M. (1961): *Preadaptive potentialities of the mammalian skull: an experiment in growth and form.* Am. J. Anat. **109**: 117–132.

EAYRS, J. T and HORN, G. (1955): *The development of cerebral cortex in hypothyroid and starved rats.* Anat. Rec. **121**: 53–61.

FREUDENBERGER, C. B. (1932): *A comparison of the Wistar Albino and the Long Evans Hybrid strain of the Norway rat.* Am. J. Anat. **50**: 293–349.

GATES, W. H. (1924–1925): *Litter size, birth weight and early growth rate of mice.* Anat. Rec. **29**: 183.

HAMMOND, J. (1932): *Pigs for pork and pigs for bacon.* J. of the Roy. Agric. Soc. of England **93**: 131–145.

HAMMOND, J. (1936): *The Physiology of milk and butter-fat secretion.* Vet. Record. **16**: 519–527.

HAMMOND, J. (1960): *Farm animals. Their breeding growth and inheritance.* 3rd edit. Edward Arnold London.

HATAI, S. (1907a): *Effect of partial starvation followed by a return to normal diets on the growth of the body and central nervous system of the albino rats.* Amer. J. Physiol. **18**: 309.

HATCH, A., BALAZS, T., WIBERG, G. S. and GRICE, H. C. (1963): *Long-term Isolation Stress in rats.* Science **142**: 507.

HENSELER, H. (1914): *Untersuchungen über den Einfluss der Ernährung auf die morphologische und physiologische Gestaltung des Tierkörpers.* Kühn Arch. Univ. Halle **5**: 207–288.

JACKSON, C. M. (1919): *The postnatal development of the suprarenal gland and the effects of inanition upon its growth and structure the Albino rat.* Am. J. Anat. **25**: 221–289.

JACKSON, C. M. (1925): *The effects of inanition and malnutrition upon growth and structure.* P. Blakiston's Son & Co. Philadelphia.

JACKSON, C. M. (1937): *Recovery of rats upon refeeding after prolonged suppression of growth by underfeeding.* Anat. Rec. **68**: 371–381.

JACKSON, C. M. and STEWART, A. C. (1920): *The effects of inanition in the young upon ultimate size of the body and of various organs in the albino rat.* J. Exp. Zool. **30**: 97.

JOHANNESSEN, L. B. (1961): *Variations in the morphology of mandibular first molars of albino rats.* J.D.R. **40**: 812–818.

JOHANNESEN, L. B. (1965): *Effects of dessicated thyroid, propylthiouracil, Vitamin B_{12} and caloric restriction on somatic growth, food intake and dentinogenesis in cortisone treated immature albino rats.* Arch. oral. Biol. **10**: 819–832.

KENNEDY, G. L. (1957a): *The effect of age on the somatic and visceral response to over nutrition in the rat.* J. Endocrin. 15. Proc. Soc. Endocrin. XIX–XXIV.

KENNEDY, G. L. (1957b): *The development with age of hypothalamic restraint upon the appetite of the rat.* J. Endocrin. **16**: 955–966.

KENNEDY, G. L. and PEARCE, W. M. (1958): *Relation between liver growth and somatic growth in the rat.* J. Endocrin. **17**: 149.

KRUGER, B. S. (1962): *Trace elements and dental morphology.* University of Queensland Papers, **1(6)**: 101–125.

MCCANCE, R. A. (1960): *Severe undernutrition in growing and adult animals. I. Production and general effects.* Brit. J. Nutr. **14**: 59–73.

MCCANCE, R. A. (1962): *Food, growth and time.* Lancet **2**: 621–626, 671–676.

MCCANCE, R. A., FORD, E. H. R. and BROWN, W. A. B. (1961): *Severe undernutrition in growing and adult animals. 7. Development of the skull, jaws and teeth in pigs.* Brit. J. Nutr. **15**: 213–224.

MCCAY, C. M., CROWELL, M. F. and MAYNARD, L. A. (1935): *The effect of retarded growth upon the length of life span and upon the ultimate body size.* J. Nutr. **10**: 63.

MCCAY, C. M., MAYNARD, L. A., SPERLING, G. and BARNES, L. L. (1939): *Retarded growth, life span, ultimate body size and age changes in the albino rat after feeding diets restricted in calories.* J. Nutr. **18**: 1.

MacDowell, E. C., Gates, W. H. and MacDowell, C. G. (1930): *The influence of the quantity of nutrition upon the growth of the suckling mouse.* Journal Gen. Physiol. **13**: 529–545.

McMeekan, C. P. (1940–1941): *Growth and development of the pig with special reference to carcass quality characters.* J. Agric. Sc. **30**: 276, 387, 511 **31**: 1.

Medawar, P. B. (1950): *Transformation of shape.* Proc. Roy. Soc. B. **13**: 433–523.

Minot, C. S. (1891): *Senesence and rejuvenation.* Journ. Physiol. **12**: 97–153.

Moment, G. B. (1933): *The effects of rate of growth on the post-natal development of the white rat.* J. Exp. Zool. **65**: 359–393.

Monod, J. and Jacob, F. (1961): *Teleonomic mechanisms in cellular metabolism, growth and differentiation.* Cold Spring Harbor Symposium on Quantitative biology. **26**: 389–401. N.Y.

Moore, W. J. (1966): *Skull growth in the albino rat.* J. Zool. Lond. **149**: 137–144.

Mortimer, H. (1937): *Pituitary and associated hormone factors in cranial growth and differentiation in the white rat: A roentgenological study.* Radiology **28**: 5–38.

Moss, M. L. (1934): *Growth of the calvaria in the rat.* Am. J. Anat. **94**: 333–359.

Moss, M. L. (1955): *Morphological changes in the growing rat skull following administration of cortisone acetate.* Proc. Soc. Exp. Biol. N.Y. **89**: 648–650.

Osborne, T. B. and Mendel, L. B. (1914): *The suppression of growth and the capacity to grow.* J. Biol. Chem. **18**: 95.

Osborne, T. B. and Mendel, L. B. (1926): *The relation of the rate of growth to diet.* J. Biol. Chem. **69**: 661.

Outhouse, J. and Mendel, L. B. (1933): *The rate of growth.* J. Exp. Zool. **64**: 257–285.

Park, E. A. (1964): *The imprinting of nutritional disturbances on the growing bone.* Pediatrics **33**: 815–862.

Park, E. A. and Richter, C. P. (1953): *Transverse lines in bone, the mechanism of their development.* Bull. Johns Hopkins Hosp. **93**: 234–248.

Parkes, A. S. (1926): *The growth of young mice according to size of litter.* Ann. Appl. Biol. **13**: 374–394.

Parkes, A. S. (1929): *Note on the growth of young mice suckled by rats.* Ann. Appl. Biol. **16**: 171–173.

Patterson, H. D. (1950): *Sampling on successive occasions with partial replacement of units.* J. Roy. Stat. Soc. B. **12**: 241.

Paynter, K. J. and Grainger, R. M. (1956): *The relation of nutrition to the morphology and size of rat molar teeth.* J. Can. Dent. Assoc. **22**: 519–531.

Paynter, K. T. and Hunt, A. M. (1964): *Morphogenesis of the rat first molar.* Arch. oral Biol. **9**: 611–626.

Prader, A., Tanner, J. M. and von Harnack, G. A. (1963): *Catch-up growth following illness or starvation; an example of developmental canalization in man.* J. Pediat. **62**: 646–659.

Prahl, B. (1968): *Sutural Growth.* M.D. Thesis: University of Nymegen, The Netherlands.

Riesenfeld, A. (1967): *Biodynamics of head form and cranio-facial relationships.* Homo. **18**: 233–251.

Reitsma, J. W. (1963): *Het merken van laboratoriumdieren.* Biotechniek **2**: 135–143.

Roubicek, C. B. (1966): *Stress adaptation in the rat as measured by postweaning growth.* Growth. **30**: 79–85.

Rugh, R.: *Effect on suckling young of X-irradiation of lactating mothers.* Proc. Soc. Exp. Biol. Med. **93**: 523–524.

Savara, B. S., Tracy, W. R. and Miller, P. A. (1966): *Analysis of errors in cephalometric measurements of 3 dimensional distances on the human mandible.* Arch. oral Biol. **11**: 209–217.

SCAMMON, R. E. (1930): *The measurement of the body in childhood* In: *The measurement of man.* HARRIS, J. A., JACKSON, C. M., PATTERSON, D. G. and SCAMMON, R. E. University of Minnesota Press.

SCOW, R. O. (1944): *Retarding effects of brief refrigeration upon skeletal development in the newborn rat.* Anat. Rec. **88:** 457 abst.

SCHOUR, I. and MASSLER, M. (1949): *The Teeth.* In: *The Rat;* ed. E. J. Farris and J. Q. Griffith, New York, Hafner.

SMITH, G. C. (1935): *The change in volume of the olfactory and accessory olfactory bulbs in the albino rat during postnatal life.* J. Comp. Neurol. **61. 3:** 477–508.

SPENCE, J. M. (1940): *Method of studying the skull development of the living rat by serial cephalometric roentgenograms.* Angle Orthodontist. **10:** 127–139.

SUGITA, N. (1918): *Comparative studies on the growth of the cerebral cortex.* J. Comp. Neur: **29:** 177–240.

SUMNER, F. B. (1915): *Some studies of environmental influence, heredity, correlation and growth, in the white mouse.* J. Exp. Zool. **18:** 325–432.

TALBERT, G. B. and HAMILTON, J. B. (1955): *The effect of reduced rate of growth on maturation of the male reproductive system of the albino rat.* Anat. Rec. **121:** 763–773.

TANNER, J. M. (1951): *Some notes on the reporting of growth data.* Hum. Biol. **23:** 93–159.

TANNER, J. M. (1962): *Growth at adolescence,* 2nd edit. Blackwell Oxford.

TANNER, J. M. (1963): *Regulation of growth.* Nature **199:** 845.

TONGE, C. H. and MCCANCE, R. A. (1965): *Severe undernutrition in growing and adult animals. 15. The mouth, jaws and teeth of pigs.* Brit. J. Nutr. **19:** 351–372.

VAN DER LINDEN, F. P. G. M. (1966): *Genetic and environmental factors in dentofacial morphology.* Am. J. Orth. **52:** 576–583.

WALLACE, L. R. (1948): *The growth of lambs and afterbirth in relation to the level of nutrition.* J. Agric. Sci. **38:** 93, 243, 367.

WALNUT, T. H. (1967): *A mathematical analysis of the changes in the size and shape of bones during growth.* Growth, **31:** 217–230.

WALTON, A. and HAMMOND, J. (1938): *The maternal effects on growth and conformation in Shire horse – Shetland pony crosses.* Proc. roy. Soc. B. **125:** 311.

WASHBURN, S. L. (1946): *The sequence of epiphyseal union in the opossum.* Anat. Rec. **95:** 353–363.

WASHBURN, S. L. (1947): *The relation of the temporalis muscle to the form of the skull.* Anat. Rec. **99:** 239–248.

WEISS, P. and KAVANAUGH, J. L. (1957): *A model of growth and growth control.* J. Gen. Physiol. **41:** 1–47.

WEIDENREICH, F. (1941): *The brain and its role in the phylogenetic transformation of the human skull.* Tr. Amer. Phyl. Soc. N.S. **31:** 321–442.

WIDDOWSON, E. M. and KENNEDY, G. L. (1962): *Rate of growth, mature weight, and life-span.* Proc. Roy, Soc. B. **156:** 96–108.

WIDDOWSON, E. M. and MCCANCE, R. A. (1960): *Some effects of accelerating growth. 1. General somatic development.* Proc. Roy. Soc. B. **152:** 188–206.

WIDDOWSON, E. M. and MCCANCE, R. A. (1963): *The effect of finite periods of undernutrition at different ages on the composition and subsequent development of the rat.* Proc. Roy. Soc. B. **158:** 329–342.

WIDDOWSON, E. M., DICKERSON, J. W. T. and MCCANCE, R. A. (1960): *Severe undernutrition in growing and adult animals. 4. The impact of severe undernutrition on the chemical composition of the soft tissues of the pig.* Brit. J. Nutr. **14:** 457–471.

WRIGHT, H. V., KJAER, I. and ASLING, C. W. (1967): *Roentgen cephalometric studies on skull development in rats.* Am. J. Phys. Anthrop. **25:** 103–118.

YATES, F. (1949): *Sampling methods for censuses and surveys.* Charles Griffin and Co. London.
YATES, F. (1950): *The place of statistics in the study of growth and form.* Proc. Roy. Soc. B. **137**: 479–488.

ACKNOWLEDGEMENTS

I acknowledge with thanks the assistance of:

Mr. H. W. B. Jansen of the Research Laboratory of the School of Dentistry, University of Nymegen, and in particular his assistant Mr. S. J. A. M. Nottet;

Mr. J. W. Reitsma and Mr. F. G. J. Janssen of the Central Animal Laboratory of the Faculty of Medicine (Head: Dr. vet. M. J. Dobbelaar). The ability and industry of Mr. Reitsma, upon which the entire experiment depended, has been expecially appreciated;

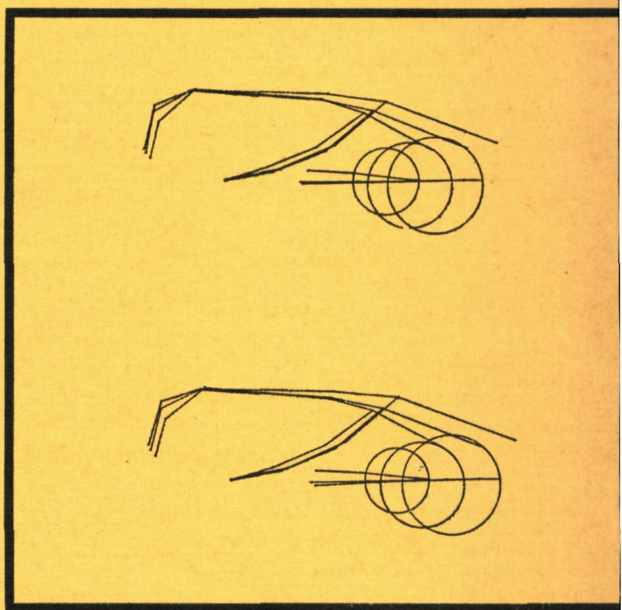
Mr. J. L. M. van de Kamp of the Section of Medical Photography (Head: Mr. A. Th. A. Reijnen);

Mr. H. C. M. Reckers of the Medical Illustrations Department (Head: Mr. C. van Huyzen);

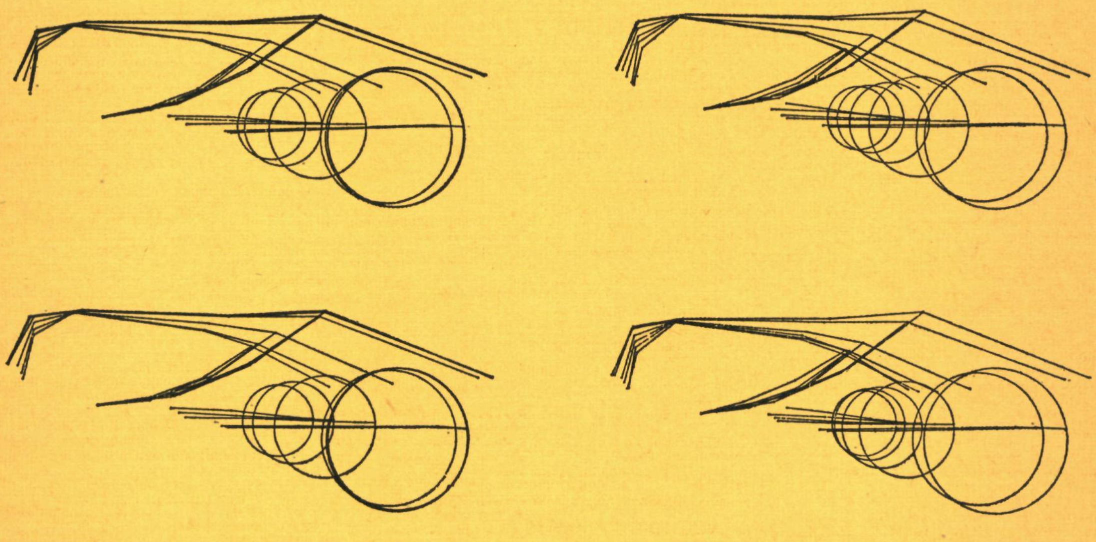
Mr. E. de Graaf, librarian of the Medical Faculty, and his assistants;

Mr. W. H. Doesberg and Mr. M. A. van 't Hof of the Mathematical Service Institute of the University of Nymegen (Head: Mr. P. van Elteren);

Miss M. E. J. Pahlplatz of the Computer Centre of the University of Nymegen.



appendix to **GROWTH PATTERN AND ENVIRONMENT**



John F. Jefferys

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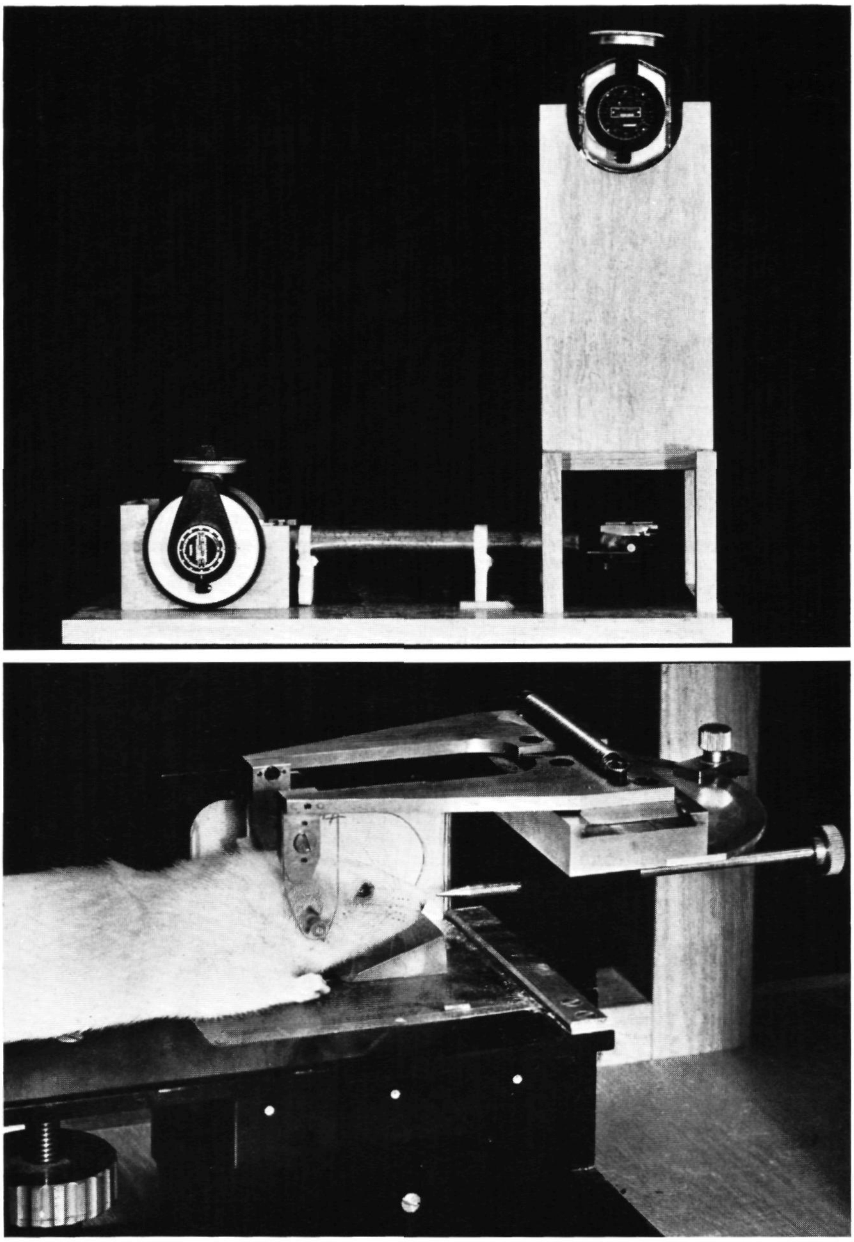
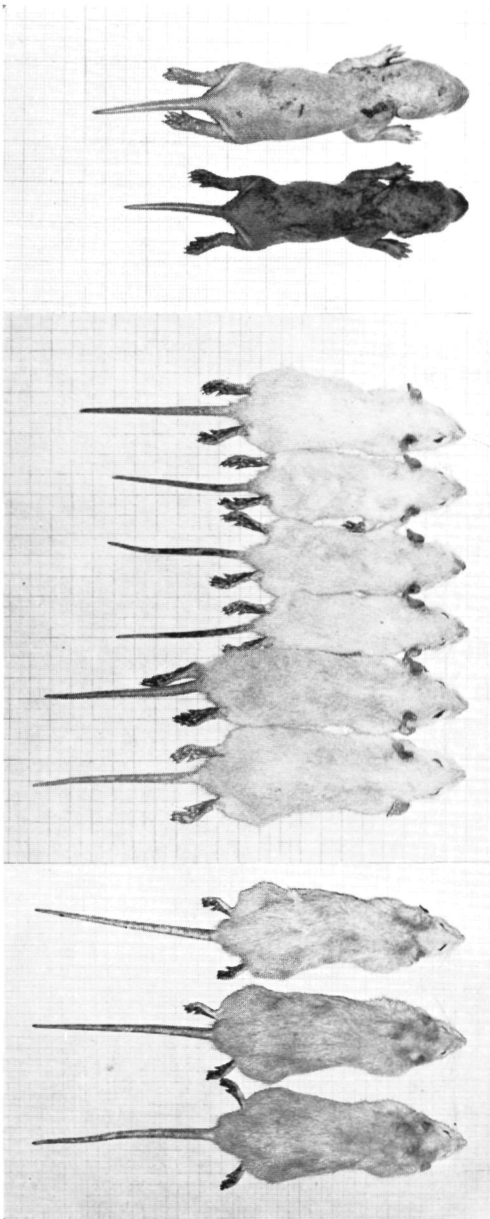


PLATE I

A. Arrangement of cephalostat and tube mountings. Cables have been removed for clarity.

B. General view of rat in cephalostat, with collimating tube removed. Plastic wedge supports mandible. Note milled table height adjustment in lower left of photograph. Nasal pin used only as indicator.

EXPERIMENT 4



Small-litter rat.

Day 30.

Large-litter rat.

Initially small-litter rats, at present in large litters.

Day 51, weaning.

Initially large-litter rats, at present in small litters.

Initially small litter rats.

Day 142.

Initially large litter rat.

PLATE II

Illustration of the superficial differences in experimental groups at different times. These are from experiment 4. The tail/body proportions clearly change with age. The darkness of the large-litter rat at day 30 is just dirt! Dimensions are indicated by millimetre squares.

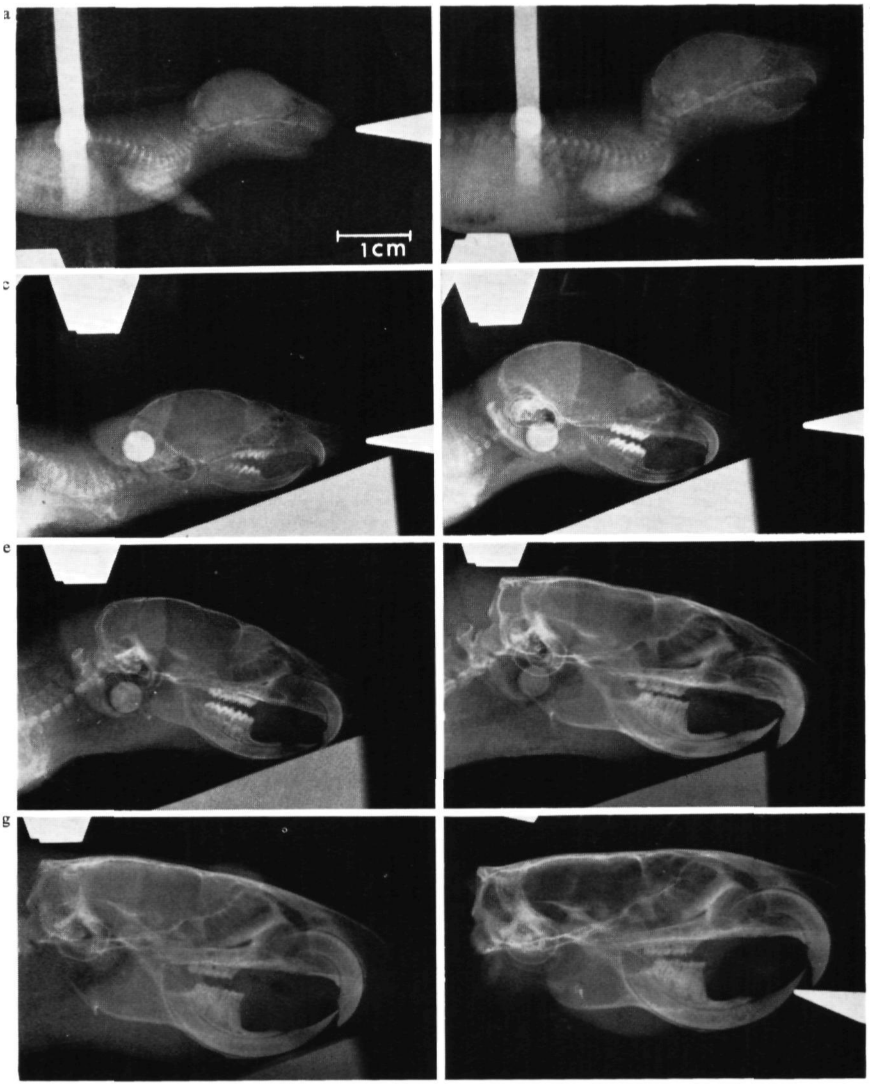


PLATE III

Typical appearance of x-ray films at stages measured. All at approximately life size. Rats from Experiment 4, selected for clarity of illustration.

a. Stage 1. At birth, 23 days P C.
This is an inverted reproduction of the original film to provide normal orientation. Landmarks are vague. Incisor and M_1 discernible.

b. Stage 2. 30 days P.C.
All landmarks relatively clear except zygomatic process of maxilla. Incisors close to eruption. M_2 just visible. Details of petrous temporal bone appearing. Fossa of paraflocculus discernible. Crista limitans undeveloped.

c. Stage 3. 37 days P.C. M_2 clear.
Incisors erupted but no attrition. Relatively retruded mandible typical of this age All landmarks clear but for zygomatic process of maxilla.

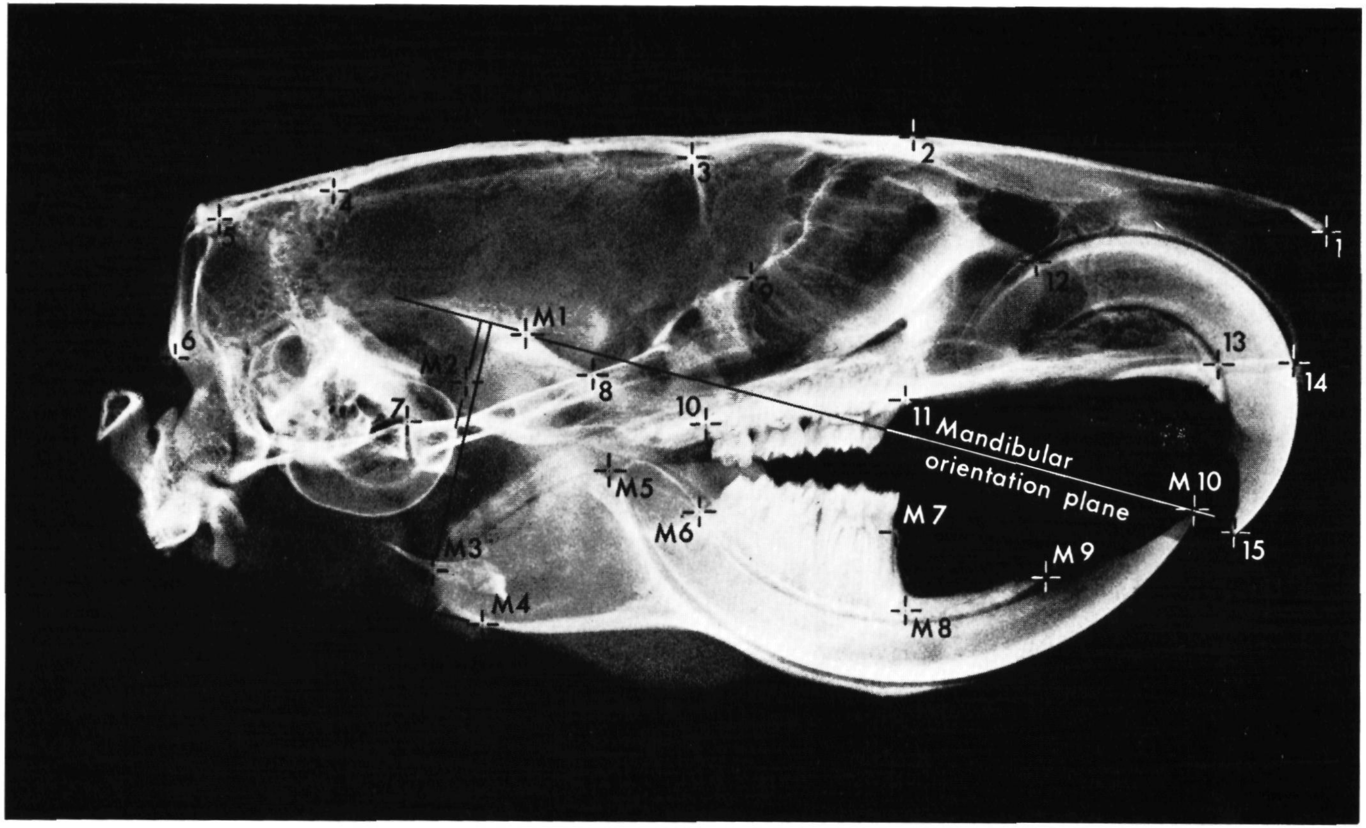
d. Stage 4. 44 days P.C. M_3 visible.
Attrition of incisors apparent Mandible now more prognathic. All landmarks visible except mandible.

e. Stage 6. 58 days P.C. M_3 in occlusion.
Tooth generating organ of maxillary incisor now caudal to zygomatic process Mandible now fairly clear. Temporal ridge visible.

f. Stage 7. 142 days P.C.
Considerable molar attrition. Fronto-nasal suture becoming obscure.

g. Stage 8. 300 days P.C.
Fronto-nasal and parieto-interparietal sutures rather obscure Ossicle at rhinion. Temporal ridge no longer visible.

h. Stage 9. 500 days P.C.
After decapitation and removing brain and skin Fronto-nasal and parieto-interparietal sutures are very obscure.



LANDMARKS USED IN MEASUREMENTS OF HARD TISSUES OF THE RAT HEAD

SKULL POINT DEFINITIONS

All images are in the median plane unless otherwise stated. When bilateral, a midpoint was used.

1. 'Rhinion'. The most anterior margin of the nasal bones. Ignore ossicles.
2. 'Nasion'. The mid-point of the external surface of the fronto-nasal suture.
3. The mid-point of the inner table at the apex of the image of the crista limitans.
4. The mid-point of the endocranial surface of the parieto-inter-parietal suture.
5. The mid-point of the endocranial surface of the occipito-inter-parietal suture Endocranial counterpart of 'inion'.
6. 'Opithion'. The most ventral point of the image of the dorsal margin of the foramen magnum.
7. The mid-point of the endocranial surface of the spheno-occipital synchondrosis.
8. The mid-point of the endocranial surface of the spheno-pres-phenoidal synchondrosis.
9. The intersection of the images of the cribriform plate and the caudal wall of the most caudad ethmoid air cell.
10. The intersection of the image of the palatal surface of the nasal floor with the bilateral images of the distal of the last maxillary molar
11. The intersection of the image of the palatal surface of the nasal floor with the bilateral images of the mesial of the first maxillary molar.
12. The intersection of the bilateral images of the anterior surfaces of the zygomatic processes of the maxillae and the enamel surfaces of the upper incisors.
13. The intersection of the images of the nasal floor and the dentinal surface of the upper incisors.
14. The intersection of the images of the nasal floor and the enamel surface of the upper incisors.
15. The apex of the image of the upper incisor incisal edges.

MANDIBULAR POINT DEFINITIONS

All images are bilateral, but in practice the few films with detectable double images were not used An orientation plane is visualised from the mandibular incisor incisal edge tangent to the mandibular condyle (In measuring the mandible this plane was lined up with the x axis of the cross-table)

M1. The point of contact of the orientation plane with the top of the mandibular condyle.

M2. The point of contact of a perpendicular from the orientation plane tangentially with the caudal surface of the mandibular condyle.

M3 The point of contact of a perpendicular from the orientation plane tangentially with the caudal surface of the angular process of the mandible.

M4. The contact point with the ventral surface of the angular process of the mandible of a tangent parallel to the orientation plane

M5 The centre of the image of the mandibular foramen.

M6. The intersection of the images of the distal of the root of the third mandibular molar and the inner curvature of the mandibular incisor

M7. The tip of the alveolar process mesial to the first mandibular molar.

M8. The centre of the image of the mental foramen.

M9. The lingual tip of the alveolar process of the mandibular incisor.

M10. The incisal edge of the mandibular incisor

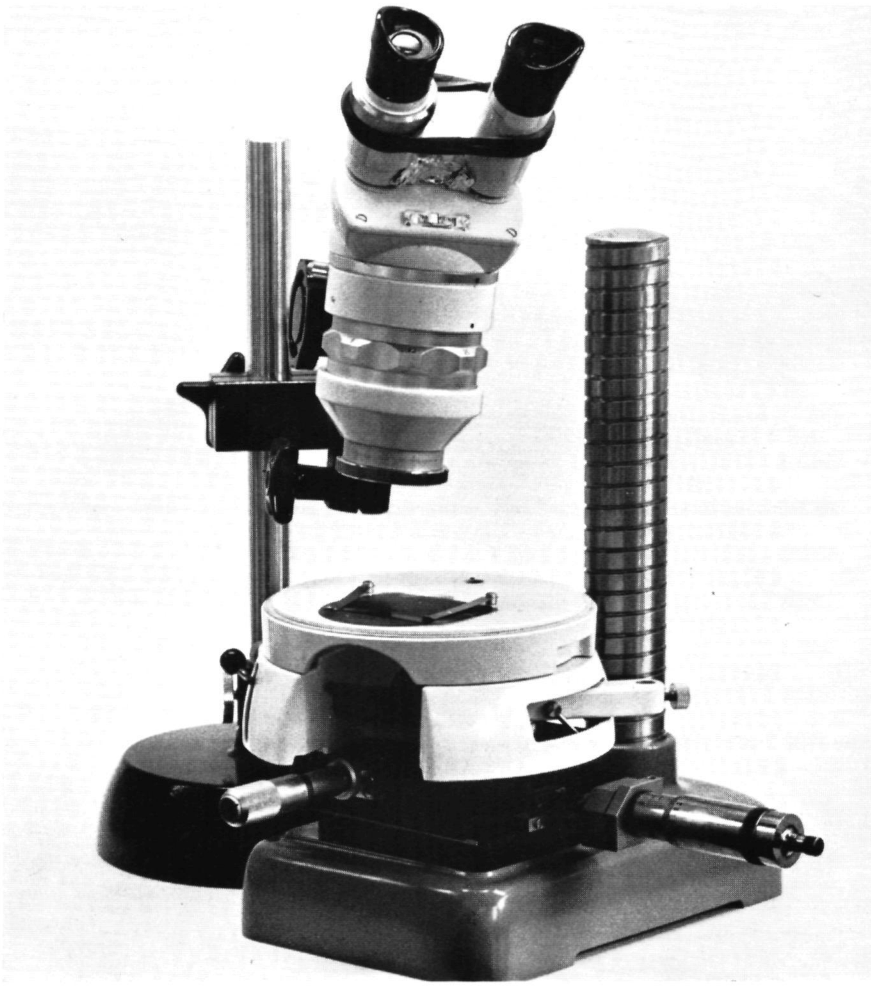


PLATE V

Arrangement of dissecting microscope transilluminating table on the micrometer-driven traversing table of the 'Durimet' microscope. Note the orientation of the body of the dissecting microscope so that the left optical path is perpendicular to the traversing table. The ocular tubes are fixed with heavy rubber bands against an interposed plastic block, to prevent shifting of the image from movement at that source. The film is held under a glass plate.

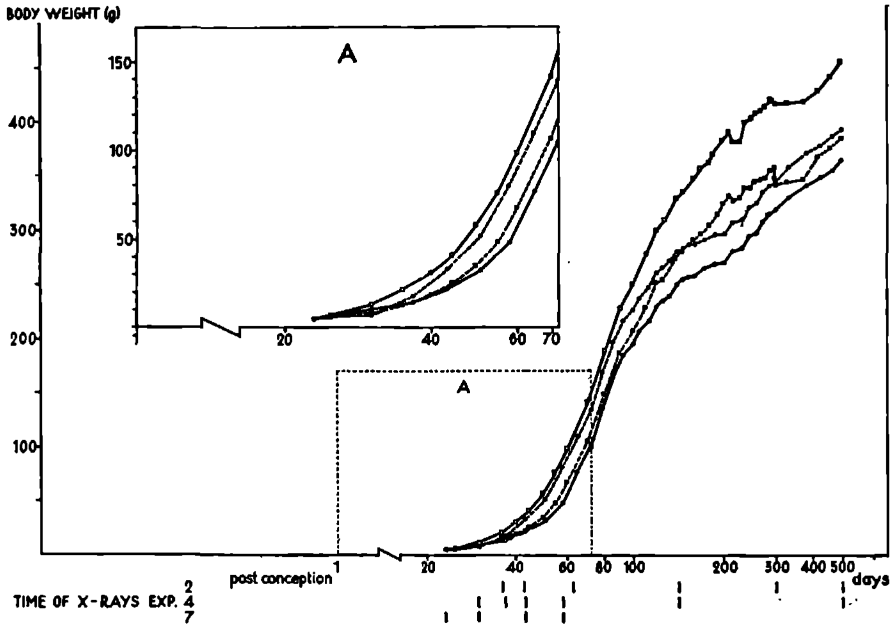


Fig. 8. Weight performance of two experimental groups of male Wistar rats (Experiment 2) superimposed on that of another two comparable groups (Experiment 4) recorded from 23 to 500 days post-conception. Experiment 2 = □ Even - ■ Odd-numbered rats
Experiment 4 = ○ Even - ● Odd-numbered rats

Inset. *Detail of earlier region of the graphs.*

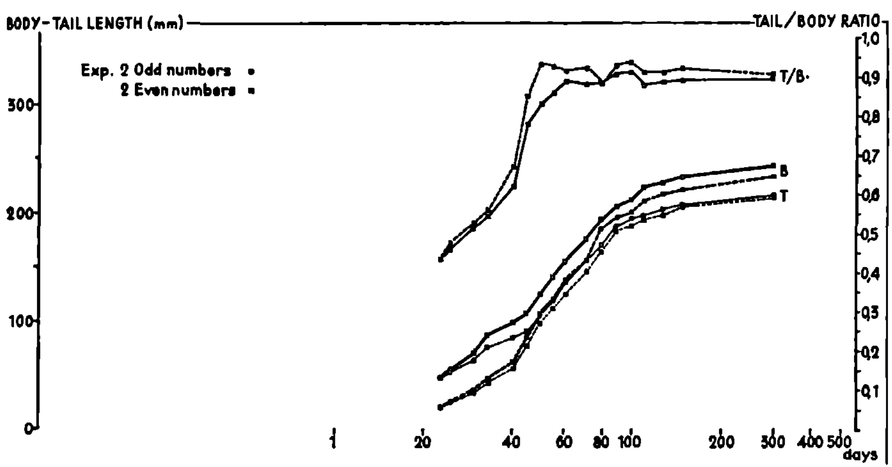


Fig. 9. Tail/Body length ratio, and tail and body length measurements, of two experimental groups of male Wistar rats (Experiment 2) recorded from 23 to 300 days post-conception.

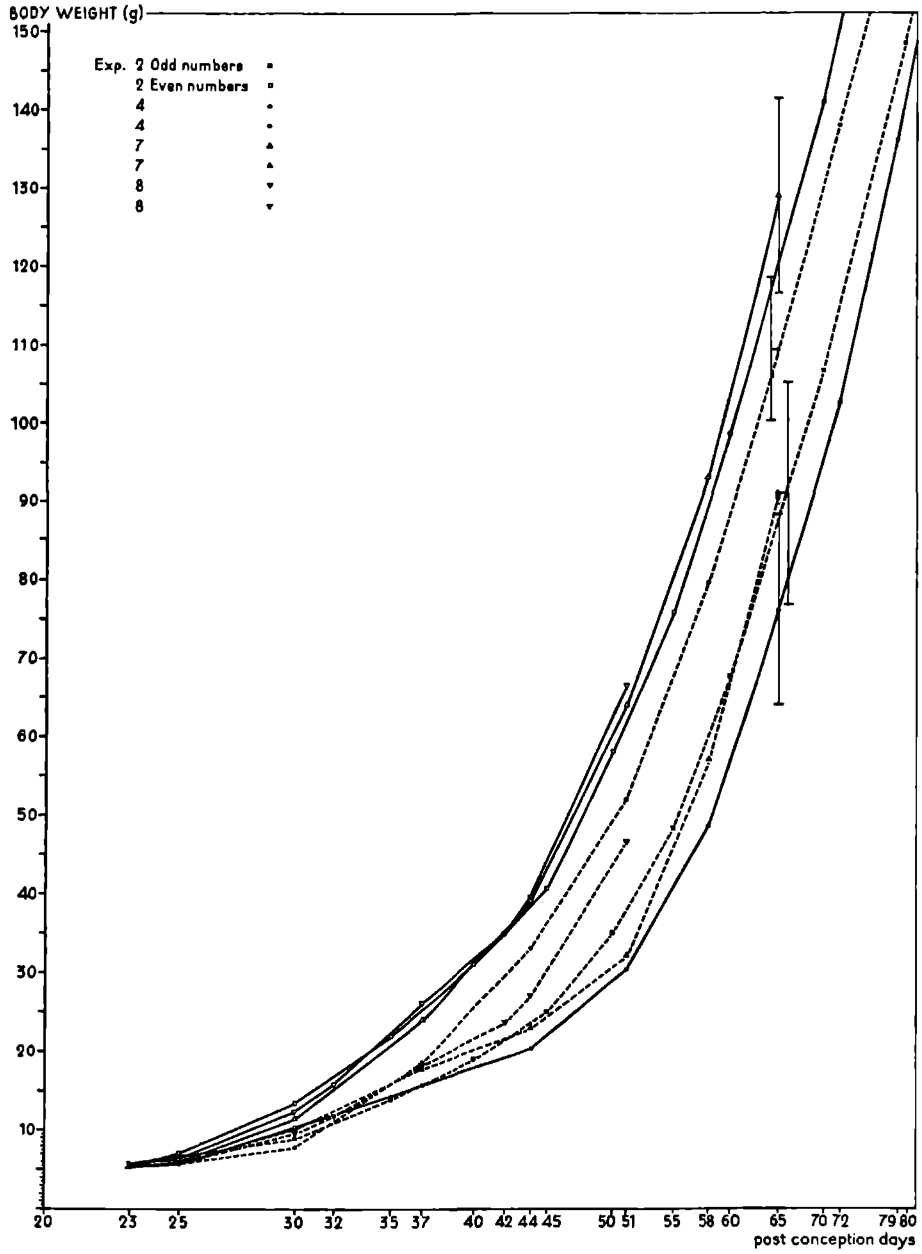


Fig. 10. Weight performance of all experimental groups of male Wistar rats used in all experiments, given according to experiment and group. Two pairs of standard deviations for the samples from Experiment 4 and Experiment 7 are given. From data recorded up to day 82 post-conception beginning at day 23 (birth). Both axes arithmetic.

Table 1 TOTAL BODY WEIGHT IN GRAMS **EXPERIMENT 2**

Time	Odd numbered rats			Even numbered rats			't' Value of Difference between Means	Probability that both samples from same population
Days Post-conception	Mean value	Standard deviation	Number in sample	Mean value	Standard deviation	Number in sample		
BIRTH								
23	5.57	0.37	34	5.52	0.34	30	-0.562	N.S.
25	6.05	0.62	34	7.05	0.63	30	6.37	***
30	8.95	0.89	32	13.31	1.53	29	13.80	***
35	13.83	1.46	30	21.91	1.88	29	18.47	***
40	18.82	2.04	30	30.94	2.56	29	20.13	***
45	24.95	3.21	30	40.60	3.28	29	18.52	***
50	34.92	5.21	30	57.93	5.18	29	17.01	***
WEANING								
55	48.02	8.79	29	75.79	7.51	29	12.93	***
60	67.60	10.05	29	98.67	8.65	29	12.55	***
70	106.45	11.73	29	140.93	12.97	28	10.53	***
80	148.23	14.18	28	187.16	17.26	28	9.22	***
90	185.57	28.97	28	226.00	19.04	28	6.17	***
100	206.04	28.45	28	249.82	31.51	28	5.46	***
110	227.40	35.20	28	276.10	22.56	27	6.08	***
120	250.20	17.17	28	299.70	24.33	27	8.74	***
130	252.6	22.38	28	308.5	24.73	27	8.80	***
140	272,4	17.91	27	327.9	26.64	27	8.98	***
150	279.1	18.72	27	333.3	24.25	27	9.19	***
160	289.0	18.25	27	345.7	24.56	27	9.63	***
170	294.4	18.68	27	353.7	24.80	27	9.92	***
180	302.6	18.19	27	360.2	23.55	27	10.06	***
190	312.2	19.09	27	368.9	23.40	27	9.76	***
200	322.4	19.16	27	380.7	22.50	27	10.25	***
210	329.3	19.27	27	388.3	23.94	27	9.98	***
220	324.0	19.32	27	379.3	21.50	27	9.94	***
230	327.5	20.16	27	390.3	21.97	27	10.94	***
240	337.2	18.08	27	397.6	21.72	27	11.10	***
250	337.4	19.61	27	400.2	22.50	27	10.93	***
260	342.0	19.55	27	405.7	23.20	27	10.91	***
270	344.6	18.55	27	407.9	24.97	27	10.57	***
280	345.5	18.30	27	411.3	24.71	27	11.12	***
290	352.1	18.75	27	418.9	26.13	27	10.79	***
300	345.9	17.09	27	414.6	29.06	27	10.59	***
340	341.7	19.50	27	413.6	28.61	26	10.73	***
380	344.3	17.98	27	415.0	31.29	26	10.13	***
420	364.4	18.87	27	426.8	33.60	26	8.37	***
460	372.9	20.58	27	439.1	38.14	26	7.90	***
500	381.5	21.35	27	451.9	34.88	25	5.74	***

N.S. = Not Significant * = $P<0.05$ ** = $P<0.01$ *** = $P<0.001$
Positive 't'-value indicates Even > Odd

Time	Odd numbered rats			Even numbered rats			't' Value of Difference between Means	Probability that both samples from same population
Days Post-conception	Mean value	Standard deviation	Number in sample	Mean value	Standard deviation	Number in sample		
BIRTH								
23	5.22	0.33	36	5.24	0.34	36	+ 0.253	N.S.
25	5.72	0.60	36	5.76	0.64	36	+ 0.274	N.S.
30	7.81	0.67	30	10.48	1.70	33	+ 8.04	***
37	18.45	2.59	30	15.65	2.25	25	— 4.23	***
44	32.9	3.5	30	20.2	2.9	25	—14.50	***
51	51.7	7.4	30	30.2	5.9	25	—11.80	***
WEANING								
58	79.7	7.1	29	48.6	9.3	21	—13.40	***
65	109.4	9.2	29	76.0	12.0	20	—11.02	***
72	137.8	11.3	29	102.6	13.4	20	— 9.91	***
79	169.9	13.1	29	135.9	13.9	20	— 8.72	***
86	194.7	15.3	29	163.0	13.3	20	— 7.52	***
93	214.6	16.0	29	183.6	12.6	20	— 7.22	***
100	224.2	16.6	29	194.2	13.5	20	— 6.69	***
107	235.5	18.3	29	205.0	14.8	20	— 6.18	***
114	246.9	20.5	29	216.0	15.5	20	— 5.69	***
121	259.6	21.2	29	228.6	17.2	20	— 5.43	***
128	263.2	22.8	29	232.9	17.7	20	— 4.99	***
135	270.7	23.1	29	238.8	18.7	20	— 5.12	***
142	278.2	23.6	29	248.2	18.6	20	— 4.75	***
149	281.6	24.5	29	252.1	20.0	20	— 4.45	***
156	284.0	24.8	29	254.6	21.1	20	— 4.33	***
163	284.7	24.7	29	256.5	19.7	20	— 4.26	***
177	289.9	24.9	29	262.5	20.7	20	— 3.91	***
191	293.4	27.0	29	267.1	21.3	20	— 3.73	***
205	294.0	28.8	29	268.9	22.3	20	— 3.27	**
219	304.3	29.9	29	279.2	23.6	20	— 3.13	**
233	307.5	31.2	29	281.2	25.0	20	— 3.14	**
247	318.6	32.6	29	292.9	27.1	20	— 2.90	**
261	322.0	35.6	29	295.0	27.8	20	— 2.84	**
275	332.4	36.3	29	305.4	30.7	20	— 2.72	**
289	338.5	36.2	29	312.9	31.0	20	— 2.57	*
303	342.1	36.3	29	316.6	30.9	20	— 2.56	*
345	355.6	38.2	29	328.8	31.3	19	— 2.55	*
387	368.9	39.7	29	339.8	34.7	19	— 2.61	*
429	375.3	40.2	29	346.3	38.6	19	— 2.48	*
471	383.5	41.4	29	352.7	39.9	19	— 2.56	*
500	389.2	38.7	28	361.3	50.7	19	— 2.14	*

N.S. = Not Significant *= $P<0.05$ **= $P<0.01$ ***= $P<0.001$
+ = Even>Odd — = Odd>Even

Table 3 TOTAL BODY WEIGHT IN GRAMS EXPERIMENT 7

Time	Odd numbered rats			Even numbered rats			't' Value of Difference between Means	Probability that both samples from same population
Days Post-conception	Mean value	Standard deviation	Number in sample	Mean value	Standard deviation	Number in sample		
BIRTH								
23	5.58	0.42	36	5.53	0.39	36	0.527	N.S.
25	5.77	0.65	33	5.94	0.75	32	0.973	N.S.
30	10.30	1.24	30	11.51	1.65	30	3.20	**
37	17.85	2.34	30	24.08	3.48	30	8.14	***
44	22.87	3.58	30	39.07	5.21	30	14.0	***
51	31.93	5.58	30	63.87	8.00	30	17.9	***
WEANING								
58	56.9	10.25	30	92.90	9.39	30	14.2	***
65	90.1	14.44	30	128.70	12.60	29	10.9	***

Table 4 TOTAL BODY WEIGHT IN GRAMS EXPERIMENT 8

Time	Odd numbered rats			Even numbered rats			't' Value of Difference between Means	Probability that both samples from same population
Days Post-conception	Mean value	Standard deviation	Number in sample	Mean value	Standard deviation	Number in sample		
BIRTH								
23	5.69	0.39	36	5.57	0.37	36	1.33	N.S.
25	5.89	0.39	35	6.31	0.72	36	3.09	**
30	9.66	0.96	28	12.43	1.81	34	7.28	***
32	11.87	1.53	27	15.76	2.16	34	7.91	***
37	18.23	2.51	26	26.18	2.39	34	12.59	***
42	23.54	2.73	26	35.07	3.59	34	13.60	***
44	27.20	3.20	26	39.50	5.1	34	10.80	***
51	46.60	3.90	14	66.30	4.9	18	12.30	***

N.S. = Not Significant * = $P<0.05$ ** = $P<0.01$ *** = $P<0.001$
Positive 't'-value indicates Even > Odd.

Table 5 BODY LENGTH IN MILLIMETRES **EXPERIMENT 2**

Time	Odd numbered rats			Even numbered rats			't' Value of	Probability that both
Days Post-conception	Mean value	Standard deviation	Number in sample	Mean value	Standard deviation	Number in sample	Difference between Means	samples from same population
BIRTH								
23	48.3	1.0	34	48.7	0.8	30	1.72	N.S.
25	52.91	2.6	34	54.90	2.0	30	3.45	**
30	63.06	2.58	32	70.28	3.22	29	9.69	***
35	75.77	4.25	30	88.24	4.03	29	11.49	***
40	83.50	4.10	30	99.10	4.71	29	13.57	***
45	90.5	4.75	30	107.97	3.60	29	15.91	***
50	105.9	6.0	30	124.6	4.1	29	13.85	***
WEANING								
55	119.9	7.2	29	140.3	5.9	29	12.83	***
60	135.6	7.2	29	153.8	5.01	29	11.17	***
70	156.5	8.1	29	175.1	5.46	28	10.13	***
80	183.4	5.8	28	192.8	5.9	28	5.99	***
90	195.5	5.07	28	205.6	4.73	28	7.71	***
100	200.6	6.10	28	211.2	5.93	28	6.59	***
110	210.0	5.76	28	222.0	6.30	27	7.37	***
130	216.9	4.38	28	228.0	3.56	27	10.30	***
150	221.5	5.26	27	232.2	3.72	27	8.63	***
300	233.7	4.99	27	242.6	5.29	27	6.36	***

Table 6 TAIL LENGTH IN MILLIMETRES **EXPERIMENT 2**

Time	Odd numbered rats			Even numbered rats			't' Value of	Probability that both
Days Post-conception	Mean value	Standard deviation	Number in sample	Mean value	Standard deviation	Number in sample	Difference between Means	samples from same population
BIRTH								
23	20.9	0.7	34	21.1	0.6	30	1.21	N.S.
25	25.24	1.05	34	25.24	0.74	29	0.00	N.S.
30	33.4	1.72	32	36.29	2.07	28	5.93	***
35	42.1	2.54	30	47.7	3.54	28	6.91	***
40	56.27	3.50	30	61.63	5.1	27	4.65	***
45	77.1	4.83	30	85.2	5.85	26	5.66	***
50	99.6	5.6	29	103.7	6.6	26	2.5	*
WEANING								
55	111.75	6.84	28	120.8	5.9	26	4.81	***
60	125.2	8.14	28	137.2	7.04	26	11.51	***
70	145.5	8.28	28	155.0	5.76	25	4.80	***
80	163.3	8.14	27	170.8	5.98	25	3.76	***
90	182.7	5.58	27	187.3	6.08	25	2.85	**
100	188.8	5.31	27	193.7	5.51	25	3.26	**
110	192.8	5.58	26	196.3	5.78	24	2.12	*
130	198.8	5.99	27	202.8	6.66	24	2.23	*
150	205.3	6.34	26	207.6	6.53	24	1.26	N.S.
300	212.9	5.82	26	215.9	6.38	24	1.74	N.S.

N.S. — Not Significant *= $P<0.05$ **= $P<0.01$ ***= $P<0.001$
Positive 't'-value indicates Even > Odd.

Table 7 TAIL/BODY LENGTH RATIO **EXPERIMENT 2**

Time	Odd numbered rats			Even numbered rats			't' Value of	Probability that both
Days Post-conception	Mean value	Standard deviation	Number in sample	Mean value	Standard deviation	Number in sample	Difference between Means	samples from same population
BIRTH								
23	.4317	.0153	34	.4333	.0130	30	+ 0.448	N.S.
25	.4773	.0154	34	.4595	.0153	29	— 4.59	* * *
30	.5299	.0215	32	.5149	.0191	28	— 2.836	*
35	.557	.021	30	.543	.035	28	— 1.842	N.S.
40	.674	.024	30	.620	.034	27	— 6.923	* * *
45	.853	.033	30	.788	.041	26	— 6.566	* * *
50	.938	.020	29	.833	.035	26	—13.82	* * *
WEANING								
55	.932	.024	28	.860	.040	26	— 8.09	* * *
60	.923	.027	28	.892	.034	26	— 3.73	* * *
70	.928	.027	28	.881	.030	25	— 6.03	* * *
80	.888	.038	27	.886	.034	25	— 0.202	N.S.
90	.934	.018	27	.910	.019	25	— 4.71	* * *
00	.938	.024	27	.916	.027	25	— 3.10	* *
10	.916	.016	26	.880	.023	24	— 6.55	* * *
30	.916	.022	27	.889	.023	24	— 4.35	* * *
50	.926	.025	26	.895	.025	24	— 4.57	* * *
00	.910	.025	26	.894	.030	25	-- 2.11	* * *

N.S.= Not Significant *= $P<0.05$ **= $P<0.01$ ***= $P<0.001$
Positive 't'-value indicates Even>Odd.

Table 8 CRANIAL CAPACITY AND ADRENAL WEIGHT; AND THEIR

Experiment 2	Body Wt. (g) at day 500			Cranial Cap in grams Hg.			Adrenal Wt. (mg)			$\frac{\text{Adr. Wt.} \times 10^5}{\text{Body Wt.}}$			Experiment 4	Body Wt. (g) at day 500		
	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n		$\bar{x}$	s.d.	n
				27.73	1.15	27	38.00	3.88	27	9.99	1.32	27				
Large-litter Rats (Odd)	381.5	21.35	27	r = 0.54*			r = 0.28						Initially large-litter (Odd)	389.2	38.7	28
p-value $H_o: r_o = 0$				$P<0.01$			$P>0.10$									
				29.18	1.39	25	43.43	6,73	25	9.59	1.45	25				
Small-litter Rats (Even)	451.9	34.88	25	r = 0.32			r = 0.19						Initially small-litter (Even)	361.3	50.7	19
p-value $H_o: r_e = 0$				$0.1<P<0.2$			$P>0.3$									

INTER-GROUP DIFFERENCES, in Body Weight, Cranial Capacity, Adrenal Weight, Relative Adrenal

	'r' Cran.Cap. /Body Wt.	'r' Adr.Wt. /Body Wt.	Body Weight	Cran.Cap. Capacity	Adr.Wt. Weight	$\frac{\text{Adr.Wt.x}10^5}{\text{Body Wt.}}$	'r' Cran.Cap. /Body Wt.	'r' Adr.Wt. /Body Wt.	Body Weight
t $H_o: \bar{x}_e - \bar{x}_o = 0$	—	—	5.74	4.19	3.62	—1.06	—	—	2.14
P $H_o: \bar{x}_e - \bar{x}_o = 0$	$P>0.30$	$P>0.20$	$P<0.001$	$P<0.001$	$P<0.001$	$0.20<P<0.40$	$P>0.10$	$P \approx 1.0$	$0.02<P<0.0$

Table 9 MAXILLARY MOLAR LENGTH, MESIO-DISTAL, MM $\times 10^{-3}$

Experiment 2 X-ray Measurement	M_1 Mean	s.d.	n	M_2 Mean	s.d.	n	M_3 Mean	s.d.	n	Total Segment Mean	s.d.	n
Odd	3466	151	28	1737	144	28	1521	126	28	6729	197	28
Even	3502	169	28	1820	158	28	1693	248	28	7017	191	28
't' Odd/Even	0.8			2.1			3.2			5.6		
'p'	>0.1			<0.05			<0.005			<0.001		
Experiment 4												
Odd	3305	163	22	2143	108	22	1465	84	17	-	-	-
Even	3374	183	15	2239	101	15	1469	171	9	-	-	-
't' Odd/Even	1.2			8.5			0.12			-	-	-
'p'	>0.1			<0.001			>0.1			-	-	-
Outhouse and Mendel				Slow Rapid			rats at about day 80			6690 6960	100 320	10 10
Paynter & Hunt	2690	80	3	(From measurement at dentino-enamel junction on enlarged reconstructions from histological sections).								

MANDIBULAR MOLAR LENGTH, MESIO-DISTAL, MM $\times 10^{-3}$

Experiment 4 X-ray Measurement	M_1 Mean	s.d.	n	M_2 Mean	s.d.	n	M_3 Mean	s.d.	n	Total Segment Mean	s.d.	n
Odd	2892	78	22	2038	83	21	1894	150	17			
Even	2994	77	15	2143	75	12	1781	96	9			
't' Odd/Even	3.8			3.66			—1.99					
'p'	<0.01			<0.01			0.05<P<0.1					

ORRELATIONS WITH BODY WEIGHT AT SACRIFICE

Cranial Cap. in grams Hg.			Adrenal Wt. (mg)			Adr.Wt. x 10^5 / Body Wt.			Experiment 7	Body Wt. (g) at day 65			Cranial Cap. in grams Hg.			Adrenal Wt. (mg)			Adr.Wt. x 10^5 / Body Wt.		
$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n		$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n
6.51	1.02	28	33.37	3.38	28	8.61	0.87	28					19.90	1.01	30	18.78	2.84	30	21.78	2.96	29
r = 0.45*			r = 0.45*						Large-litter Rats (Odd)	90.1	14.44	30	r = 0.70*			r = 0.63*					
P<0.02			0.01<P<0.02										P<0.01			P<0.01					
5.62	1.11	19	33.12	4.47	18	9.18	1.35	18					21.62	1.44	25	25.29	3.41	25	19.71	2.64	25
r = —0.39			r = 0.45						Small-litter Rats (Even)	128.70	12.60	29	r = 0.71*			r = 0.48*					
P>0.8			0.1>P>0.05										P<0.01			P<0.02					

eight and the Correlations between Body Weight and Cranial Capacity, and Body Weight and Adrenal Weight.

Cranial Capacity	Adrenal Weight	Adr.Wt. x 10^5 / Body Weight	'r' Cranial /Body Wt.	'r' Adrenal /Body Wt.	Body Weight	Cranial Capacity	Adrenal Weight	Adr.Wt. x 10^5 / Body Wt.
2.85	0.21	1.90	—	—	10.9	5.30	7.73	—2.69
P<0.01	P>0.80	0.05<P<0.10	P>0.90	P>0.40	P<0.001	P<0.001	P<0.001	P<0.01

Table 9 *Continued*

Experiment 7 Direct Measurement	M_1			M_2			M_3			Total Segment		
	Mean	s.d.	n	Mean	s.d.	n	Mean	s.d.	n	Mean	s.d.	n
Odd	2913	78	30	2029	57	30	1948	77	30	6890	156	30
Even	2950	53	29	2046	69	29	2011	90	29	7000	151	29
't' Odd/Even	2.1			1.1			2.9			2.8		
'p'	0.02<p<0.05			>0.1			<0.01			<0.01		
Johannessen	3080	80	106	(From measurement of maximum mesio-distal width on sectioned molars from Sprague-Dawley albino rats).								

MANDIBULAR MOLAR WIDTH, BUCCO-LINGUAL, MM × 10^{-3}

Experiment 7 Direct Measurement	M_1			M_2			M_3			Total Segment		
	Mean	s.d.	n	Mean	s.d.	n	Mean	s.d.	n	Mean	s.d.	n
Odd	1823	60	30	1909	71	30	1564	35	30			
Even	1853	64	29	1957	56	29	1620	51	29			
't' Odd/Even	1.93			2.9			5.1					
'p'	0.05<p<0.1			<0.01			<0.01					
Johannessen	1870	140	91									

Correlation 'r' of length & width

	M_1	M_2	M_3
Odd	0.628	0.174	0.156
Even	0.414	0.340	0.534
Odd	<0.01	>0.1	>0.1
Even	0.02<p<0.05	0.05<p<0.1	<0.01

Table 10 INITIALLY LARGE-LITTER RATS (ODD)

Rat no.	EXPERIMENT 2 Stage 3	4	6	7	8	9	EXPERIMENT 4 Stage 2	3	4	6	7	9	EXPERIMENT 7 Stage 1	2	4	6
1	*		*	*	*	*	*	*	*		*	*	*	*	*	*
3	*				*	*	*			*	*	*	*	*		
5	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
7	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*
9	*	*	*	●	●	●		*	*	*		*		*	*	*
11		*	*	*	*	*	*	*	*	*	*	*		*	*	*
13	*		*	*	*		*	*	*	*		*		*	*	*
15	*	*	*	*		*								*		*
17	*		*	*		*										
19	*	*	*	*	*	*					*	*		*		*
21			*	*	*	*	*	*	*	*	*	*		*		*
21			*	*	*	*	*	*	*	*	*	*		*	*	*
23							*	*		*	*			*	*	*
25	*		*	*	*	*						*	*			
27	*	*	*		*	*	*	*		*	*	*		*		
29		*			*	*					*	*		*	*	*
31		*					*		*	*	*		*	*	*	*
33	*	*		*											*	*
35		*	*	*	*	*					*	*	*	*	*	*
37	*	*		*	*	*	*	*	*		*		*	*	*	*
39			*	*	*	*	*	*	*	*	*	*	*			
41		*	*	*	*	*				*	*			*		
43		*	*	*	*								*	*	*	
45							*	*	*		*	*			*	*
47		*		*	*	*	*	*	*	*	*	*		*		*
49	*	*	*	*	*	*	*	*	*	*		*				
51							*	*	*	*	*	*		*	*	*
53	*	*					*	*	*	*		*				
55							*	*			*			*	*	
57	*	*		*	*										*	*
59		*		*	*	*	*	*	*	*	*	*		*	*	
61	*	*	*	*			*									
63	*		*	*	*	*	*			*	*	*			*	*
65	*	*	*	*	*	*	*		*							*
67	*	*	*	*	*	*		*	*	*	*	*			*	*
69															*	*
71							*	*	*	*	*				*	*
L_h		14	13	19	20	20		18	17	15	14	15		8	16	20
C_h	20	8	8	5	3	2	23	2	2	5	7	7	10	14	7	4
N	20	22	21	24	23	22	20	20	19	20	21	22	10	22	23	24

PRESENCE OR ABSENCE OF RATS IN THE X-RAY RECORLS USED FOR THE VARIOUS STAGES IN THE CEPHALOMETRIC STUDY.

INITIALLY SMALL-LITTER RATS (EVEN)

Rat no.	Stage (EXPERIMENT 2)	3	4	6	7	8	9	Stage (EXPERIMENT 4)	2	3	4	6	7	9	Stage (EXPERIMENT 7)	1	2	4	6
2									*							*		*	*
4				*	*	*	*		*		*	*	*			*	*	*	*
6				*	*	*	*		*	*	*	*	*	*		*	*	*	*
8		*	*	*	*	*			*							*	*	*	*
10		*		*	*	*	*		*	*	*	*	*				*	*	*
12		*	*	*	*	*	*		*	*	*						*	*	*
14		*	*	*	*	*	*			*	*	*	*	*			*	*	
16		*	*	*	*	*	*		*								*		*
18		*	*	*	*				*	*	*	*		*			*	*	
20		*	*		*	*	*		*	*	*						*	*	*
22		*	*	*			*		*	*	*	*	*	*				*	*
64		*			*		*									*	*	*	*
26			*	*			*						*	*			*	*	
28		*	*	*	*	*	*		*	*	*	*	*	*					*
30			*		*		*						*	*			*	*	*
32		*	*		*	*	*									*	*	*	*
34		*								*	*			*					*
36		*	*	*		*	*			*		*		*		*	*		*
38		*	*						*							*			
38		*	*						*							*			
40			*	*	*	*	*									*			
42			*	*	*	*	*			*	*	*	*	*			*	*	*
44		*		*	*	*			*	*	*	*				*	*	*	*
46		*		*	*	*	*												
48		*	*		*	*	*		*	*	*	*		*					
50		*	*	*	*	*	*						*	*					
52		*		*	*	*			*	*		*	*	*			*		
54		*		*		*	*		*	*	*								
56		*	*			*	*						*	*					
58		*	*	*			*		*									*	*
60		*	*		●	●							*	*				*	*
62									*										*
64									*	*		*	*	*				*	*
66									*	*	*	*	*	*				*	*
68									*								*	*	*
70																	*	*	*
72									*	*	*	*	*	*			*		*
L_h			15	13	15	17	17			14	15	12	11	14			7	16	19
C_h		22	5	7	5	3	5		22	4	1	3	5	4		10	43	6	6
N		22	20	20	20	20	22		22	18	16	15	16	18		10	20	22	25

Nos. 9 and 60 not counted when ● (ill).

L_h is the number present that are also present in the previous stage.
C_h is the number present that are not present in the previous stage.
N is the total sample number.
The same numbers were used to identify rats in all experiments, but no rat appears in more than one experiment.

Tables 11 to 59 give the inter-stage pure longitudinal incremental data for selected quantities for experiments 2, 4, and 7. This includes the mean at stage 6 only. Indications of 'p' values are given for two hypotheses tested by the Student test: the first being that there is no inter-group difference between the mean increments, and the second is that there is no difference between the mean actual dimensions measured. Except for angles, relative increments are also given.

Means at other stages than stage 6 may be found by adding or subtracting the increments to the required stage. The standard deviation of that mean can be derived from the 't' value if the standard deviations of both groups are assumed to be alike. It should be borne in mind that being longitudinal the incremental sample numbers are sometimes considerably smaller than those of the whole sample, as can be seen from the table 10.

Distances are given in millimetres, areas in square millimetres, and angles in degrees. The groups are denoted as odd (large-litter) and even (small-litter) rats. In the case of Experiment 4 this refers to the litter-size before the change-over at day 30. (Stage 2).

The statement of the nul hypothesis above the tables is over-abbreviated. It is in reality H_o: $x_e - x_o = 0$. Thus a negative t-value indicates that the 'odd' mean is greater than the 'even'.

* = $p < 0.05$ ** = $p < 0.01$ *** = $p < 0.001$

Where increments are not between successive stages, the relevant stages are indicated by a line.

Stage 5 does not exist in these records.

Table 11	EXPERIMENT 2								EXPERIMENT 4								EXPERIMENT 7							
'x' Distance 1–2 (mm) Inter-stage Longitudinal INCREMENTS 'Δx'	Even			Odd			H_o: Δx_e = = Δx_o	H_o: x_e = = x_o	Even			Odd			H_o: Δx_e = = Δx_o	H_o: x_e = = x_o	Even			Odd			H_o: Δx_e = = Δx_o	H_o: x_e = = x_e
Stage no.	Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t	Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t	Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t
1								−1.30								−1.30								−1.30
	3.84	0.16	0.88	3.23	0.31	0.74	4.28***		1.35	0.04	0.32	11.01	0.25	0.22	3.18**		1.93	0.07	0.45	1.67	0.16	0.37	3.79**	
2																3.40**								0.20
									1.81	0.08	0.31	2.22	0.09	0.41	−12.08***		4.12	0.13	0.66	3.44	0.09	0.55	16.54***	
3								4.65***								0.07								
	1.90	0.13	0.23	1.68	0.09	0.22	5.01***		1.58	0.10	0.20	2.16	0.05	0.28	−19.36***									
4								5.59***								−4.22***								4.64***
	2.96	0.15	0.29	3.03	0.10	0.33	−1.48		2.02	0.11	0.22	2.60	0.11	0.26	−13.01***		2.35	0.09	0.22	1.97	0.07	0.20	14.28***	
6								7.31***								−6.98***								8.85***
	4.19	0.08	0.32	4.76	0.12	0.39	−15.82***		5.94	0.19	0.52	4.98	0.11	0.40	15.00***									
7								3.33**								−0.95								
	1.43	0.10	0.08	1.38	0.10	0.08	1.56		1.25	0.13	0.07	1.32	0.12	0.07	−1.49									
8								3.01**																
	0.30	0.16	0.01	0.13	0.21	0.00	2.67*																	
9								2.33*								−0.09								

Table 12

EXPERIMENT 2

'x' Distance 1–6 (mm)	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								-1.86
		11.86	0.28	0.71	10.02	0.33	0.59	10.36***	
	2								
	3								8.61***
		3.91	0.09	0.13	3.50	0.07	0.13	12.67***	
	4								11.02***
		6.05	0.11	0.18	6.37	0.13	0.21	-6.61***	
	6								9.79***
		9.39	0.10	0.24	10.42	0.19	0.28	-18.48***	
	7								6.21***
		2.61	0.12	0.05	2.79	0.09	0.05	-4.99***	
	8								5.02***
		0.82	0.10	0.01	0.63	0.04	0.01	7.90***	
	9								5.55***
Stage 6 Size 'x'		38.56	0.58		36.20	0.91			

EXPERIMENT 4

'x' Distance 1–6 (mm)	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								-1.86
		5.31	0.23	0.32	4.20	0.29	0.25	7.38***	
	2								4.75
		4.36	0.15	0.19	5.66	0.17	0.27	-22.34***	
	3								0.15
		3.13	0.12	0.11	4.95	0.07	0.18	-50.45***	
	4								-5.89***
		4.16	0.22	0.14	5.11	0.10	0.16	-14.57***	
	6								-9.62***
		12.36	0.28	0.36	10.08	0.17	0.27	24.73***	
	7								-2.95**
		3.46	0.12	0.07	2.97	0.08	0.06	12.13***	
	8								
	9								-1.29
Stage 6 Size 'x'		33.66	1.22		36.56	0.50			

EXPERIMENT 7

'x' Distance 1–6 (mm)	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								-1.86
		6.15	0.22	0.36	6.11	0.18	0.36	0.38	
	2								0.30
		9.99	0.15	0.44	8.27	0.05	0.36	41.00***	
	3								
	4								7.45***
		4.84	0.07	0.14	4.07	0.14	0.13	21.03***	
	6								11.60***
Stage 6 Size 'x'		37.64	0.71		35.14	0.81			

Table 13

EXPERIMENT 2

'x' Distance 1–3 (mm)	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								-1.38
		9.16	0.14	0.75	7.92	0.32	0.64	8.62***	
	2								
	3								8.24***
		3.40	0.08	0.15	3.03	0.08	0.15	11.48***	
	4								9.80***
		5.37	0.10	0.21	5.53	0.10	0.24	-4.02***	
	6								9.76***
		8.46	0.10	0.27	9.50	0.17	0.33	-20.45***	
	7								4.84***
		2.35	0.11	0.06	2.48	0.10	0.06	-3.82***	
	8								4.31***
		0.67	0.07	0.01	0.51	0.06	0.01	6.66***	
	9								5.47***
Stage 6 Size 'x'		30.31	0.50		28.28	0.78			

EXPERIMENT 4

'x' Distance 1–3 (mm)	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								-1.38
		4.11	0.22	0.34	3.19	0.26	0.26	6.68***	
	2								4.99***
		3.58	0.10	0.21	4.52	0.13	0.29	-21.95***	
	3								0.71
		2.45	0.10	0.12	3.99	0.04	0.20	-55.43***	
	4								-5.39***
		3.70	0.20	0.16	4.48	0.08	0.18	-13.47***	
	6								-8.78***
		11.28	0.25	0.43	9.38	0.16	0.33	22.41***	
	7								-1.98
		3.10	0.10	0.08	2.81	0.07	0.07	8.29***	
	8								-0.94
	9								
Stage 6 Size 'x'		26.00	1.05		28.30	0.44			

EXPERIMENT 7

'x' Distance 1–3 (mm)	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								-1.38
		4.82	0.16	0.39	3.74	0.17	0.38	0.90	
	2								0.53
		8.04	0.13	0.47	6.58	0.06	0.39	40.04***	
	3								
	4								6.86***
		4.41	0.06	0.17	3.74	0.11	0.15	22.48***	
	6								11.08***
Stage 6 Size 'x'		29.42	0.63		27.30	0.72			

Table 14 — 'x' Distance 1–14 (mm); Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								0.70
	1.01	0.09	0.44	0.89	0.11	0.41	1.99	
2								
3								4.27***
	0.37	0.09	0.11	0.45	0.04	0.15	−2.83**	
4								4.02***
	0.60	0.05	0.16	0.63	0.04	0.18	−1.89	
6								6.10***
	1.23	0.03	0.28	1.29	0.03	0.31	−5.41***	
7								3.98***
	0.31	0.04	0.05	0.45	0.03	0.08	−10.86***	
8								0.36
	0.06	0.06	0.01	−0.05	0.05	−0.00	6.07***	
9								2.33*
Stage 6 Size 'x'	4.33	0.10		4.08	0.14			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								0.70
	0.59	0.14	0.27	0.27	0.06	0.12	5.17***	
2								4.27***
	0.40	0.04	0.15	0.62	0.03	0.25	−15.12***	
3								−0.74
	0.17	0.05	0.05	0.37	0.04	0.12	−11.56***	
4								−3.85***
	0.53	0.04	0.16	0.65	0.03	0.19	−7.60***	
6								−7.08***
	1.67	0.04	0.45	1.33	0.04	0.32	17.66***	
7								−0.49
	0.35	0.06	0.06	0.37	0.02	0.06	−0.77	
8								
9								−0.79
Stage 6 Size 'x'	3.68	0.21		4.10	0.12			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								0.70
	0.53	0.10	0.24	0.48	0.07	0.21	1.20	
2								1.46
	0.90	0.07	0.33	0.76	0.03	0.29	6.79***	
3								
4								4.82***
	0.61	0.03	0.16	0.54	0.03	0.03	6.73***	
6								6.28***
Stage 6 Size 'x'	4.25	0.18		3.94	0.14			

Table 15 — 'x' Distance 2–3 (mm); Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								0.84
	2.07	0.08	0.82	1.98	0.12	0.79	1.43	
2								
3								4.81***
	0.70	0.10	0.15	0.47	0.06	0.10	7.22***	
4								5.97***
	1.54	0.11	0.28	1.38	0.09	0.29	3.97***	
6								9.24***
	2.38	0.08	0.34	2.56	0.07	0.41	−6.22***	
7								2.92***
	0.42	0.14	0.04	0.63	0.07	0.07	−5.66***	
8								3.22**
	0.40	0.19	0.04	0.43	0.20	0.04	−0.46	
9								1.71

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								0.84
	0.97	0.22	0.37	1.10	0.12	0.47	−1.36	
2								1.89
	0.60	0.08	0.16	0.70	0.06	0.20	−3.67**	
3								1.33
	0.33	0.05	0.07	0.80	0.05	0.19	−23.13***	
4								−3.63***
	0.95	0.08	0.20	1.13	0.08	0.23	−5.72***	
6								−5.64***
	3.02	0.15	0.55	2.41	0.08	0.39	12.61***	
7								−1.53
	1.32	0.13	0.15	0.96	0.11	0.11	7.84***	
8								
9								−0.60

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								0.84
	0.87	0.10	0.34	1.18	0.14	0.47	−4.55***	
2								−1.35
	2.00	0.17	0.58	1.41	0.04	0.40	12.95***	
3								
4								3.29**
	1.20	0.17	0.22	0.90	0.06	0.17	7.36***	
6								7.24***

Table 16

'x' Distance 2–5 (mm) — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								–0 00
	8 30	0 26	0 69	7 03	0 14	0 58	10 37***	
2								
3								9 77***
	1 79	0 10	0 08	1 73	0 06	0 09	1 59	
4								8 04***
	2 74	0 12	0 12	2 97	0 11	0 13	–0 97	
6								9 36***
	4 72	0 16	0 18	5 11	0 12	0 21	–7 75***	
7								4 28***
	0 95	0 14	0 03	1 11	0 08	0 03	–4 07***	
8								3 93***
	0 48	0 22	0 01	0 49	0 21	0 01	–0 15	
9								2 26*
Stage 6 Size 'x'	24 91	0 37		23 59	0 51			

EXPERIMENT 4

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								–0 00
	4 23	0 22	0 35	3 79	0 11	0 31	4 48***	
2								3 70***
	2 68	0 08	0 16	3 47	0 09	0 22	24 83***	
3								–0 16
	1 27	0 10	0 06	2 26	0 08	0 11	–30 30***	
4								–5 28***
	1 73	0 14	0 08	2 10	0 10	0 09	–7 93***	
6								–7 99***
	6 03	0 19	0 27	4 70	0 12	0 19	21 06***	
7								–1 86
	2 01	0 11	0 07	1 55	0 13	0 05	9 82***	
8								
9								–0 54
Stage 6 Size 'x'	22 14	0 62		23 61	0 46			

EXPERIMENT 7

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_e$ t
1								–0 00
	4 35	0 25	0 35	4 83	0 16	0 40	–4 39***	
2								–0 40
	5 63	0 13	0 34	4 70	0 08	0 28	23 29***	
3								
4								6 09***
	2 20	0 08	0 10	1 82	0 10	0 08	15 56***	
6								8 80***
Stage 6 Size 'x'	24 30	0 45		23 08	0 51			

Table 17

'x' Distance 2–6 (mm) — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								–1 39
	8 25	0 16	0 63	6 92	0 14	0 51	15 02***	
2								
3								10 56***
	1 95	0 09	0 09	1 75	0 07	0 08	6 17***	
4								11 50***
	3 28	0 12	0 13	3 52	0 11	0 16	–5.42***	
6								9 77***
	5 46	0 11	0 20	5 98	0 12	0 23	–12 23***	
7								4 69***
	1 28	0 15	0 03	1 51	0 08	0 04	–5 76***	
8								4 12***
	0 70	0 20	0 02	0 68	0 20	0 02	0 32	
9								2 61*
Stage 6 Size 'x'	26 72	0 37		25 27	0 55			

EXPERIMENT 4

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								–1 39
	4 09	0 21	0 31	3 37	0 10	0 25	7 76***	
2								4 31***
	2 50	0 10	0 14	3 43	0 10	0 20	–25 59***	
3								–0 07
	1 56	0 09	0 07	2 73	0 08	0 13	–37 34***	
4								–6 61***
	2 16	0 13	0 10	2 63	0 08	0 11	–11 11***	
6								–9 56***
	6 82	0 17	0 28	5 40	0 10	0 21	25 17***	
7								–3 21**
	2 58	0 13	0 08	1 95	0 12	0 06	12 90***	
8								
9								–0 99
Stage 6 Size 'x'	23 59	0 69		25 38	0 40			

EXPERIMENT 7

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_e$ t
1								–1 39
	4 43	0 20	0 33	4 54	0 15	0 34	–1 22	
2								0 16
	5 80	0 11	0 33	4 74	0 07	0 27	31 87***	
3								
4								7 42***
	2 71	0 06	0 11	2 23	0 09	0 09	18 52***	
6								10 73***
Stage 6 Size 'x'	26 06	0 48		24 56	0 50			

Table 18

'x' Distance 2–7 (mm)	Stage no.	EXPERIMENT 2								EXPERIMENT 4								EXPERIMENT 7							
		Even			Odd			$H_0: \Delta x_e = \Delta x_o$	$H_0: x_e = x_o$	Even			Odd			$H_0: \Delta x_e = \Delta x_o$	$H_0: x_e = x_o$	Even			Odd			$H_0: \Delta x_e = \Delta x_o$	$H_0: x_e = x_e$
		Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t	Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t	Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								–0.35								–0.35								–0.35
		5.69	0.09	0.60	4.98	0.13	0.53	10.22***		2.90	0.15	0.31	2.38	0.08	0.25	7.76***		3.18	0.14	0.34	3.24	0.11	0.34	–0.99	
	2																4.63***								0.60
										1.82	0.07	0.14	2.44	0.06	0.21	–25.91***		4.06	0.12	0.32	3.26	0.06	0.26	23.16***	
	3								10.01***								0.61								
		1.58	0.10	0.10	1.41	0.05	0.09	5.68***		1.05	0.06	0.07	1.96	0.05	0.14	–44.69***									
	4								10.32***								–6.22***								5.68***
		2.88	0.11	0.17	3.04	0.09	0.19	–4.00***		1.87	0.11	0.12	2.24	0.06	0.14	–10.37***		2.56	0.08	0.15	2.08	0.13	0.13	19.77***	
	6								9.74***								–8.76***								10.82***
		4.59	0.11	0.23	5.13	0.11	0.27	–13.68***		5.95	0.16	0.34	4.84	0.09	0.26	21.27***									
	7								3.06**								–2.23*								
		1.14	0.12	0.04	1.31	0.09	0.05	–4.65***		2.35	0.12	0.10	1.92	0.12	0.08	9.40***									
	8								2.97**																
		0.57	0.16	0.02	0.60	0.18	0.02	–0.44																	
	9								2.03*								–0.61								
Stage 6 Size 'x'		19.66	0.27		18.53	0.44				16.99	0.56		18.32	0.32				19.05	0.39		17.78	0.43			

Table 19

'x' Distance 2–8 (mm)	Stage no.	EXPERIMENT 2								EXPERIMENT 4								EXPERIMENT 7							
		Even			Odd			$H_0: \Delta x_e = \Delta x_o$	$H_0: x_e = x_o$	Even			Odd			$H_0: \Delta x_e = \Delta x_o$	$H_0: x_e = x_o$	Even			Odd			$H_0: \Delta x_e = \Delta x_o$	$H_0: x_e = x_e$
		Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t	Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t	Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								–0.16								–0.16								–0.16
		4.10	0.11	0.60	3.58	0.14	0.53	6.66***		2.22	0.15	0.33	1.62	0.15	0.23	7.00***		2.25	0.15	0.33	2.50	0.10	0.37	–3.76**	
	2																4.81***								0.69
										1.23	0.06	0.13	1.76	0.06	0.21	–24.40***		2.68	0.13	0.29	2.25	0.07	0.25	11.36***	
	3								10.60***								0.25								
		0.97	0.08	0.08	0.97	0.04	0.09	–0.03		0.79	0.06	0.07	1.32	0.04	0.13	–28.24***									
	4								7.15***								–4.80***								3.92***
		1.87	0.09	0.15	1.92	0.07	0.17	–1.42		1.14	0.08	0.10	1.42	0.05	0.12	–10.18***		1.73	0.09	0.14	1.30	0.05	0.11	17.79***	
	6								9.30***								–8.05***								9.69***
		2.99	0.12	0.21	3.24	0.07	0.24	–7.25***		3.79	0.14	0.31	2.97	0.09	0.22	17.53***									
	7								2.46*								–1.09								
		0.41	0.11	0.02	0.70	0.09	0.04	–8.48***		1.43	0.14	0.09	1.12	0.13	0.07	6.17***									
	8								1.07																
		0.48	0.15	0.02	0.28	0.17	0.01	3.79***																	
	9								2.08*								–0.55								

Table 20

'x' Distance 2–9 (mm). Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								0.96
1–3	1.67	0.07	0.43	1.56	0.23	0.41	1.11	
3								5.98***
3–4	0.76	0.09	0.13	0.68	0.06	0.13	2.74*	
4								3.6***
4–6	1.01	0.10	0.15	0.92	0.08	0.15	2.22*	
6								6.02***
6–7	1.58	0.10	0.21	1.68	0.06	0.24	−3.41**	
7								2.99**
7–8	0.36	0.08	0.04	0.54	0.06	0.06	−7.58***	
8								1.55
8–9	0.30	0.13	0.03	0.23	0.14	0.02	1.40	
9								1.53
Stage 6 Size 'x'	7.38	0.14		6.97	0.27			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								0.96
1–2	0.48	0.12	0.12	0.34	0.16	0.09	1.73	
2								1.89
2–3	0.83	0.09	0.18	0.94	0.06	0.21	−3.93***	
3								1.49
3–4	0.44	0.04	0.08	0.75	0.03	0.14	−23.87***	
4								−3.77***
4–6	0.66	0.07	0.11	0.90	0.04	0.15	−10.94***	
6								−6.64***
6–7	2.05	0.10	0.31	1.68	0.07	0.24	10.35***	
7								−2.17*
7–9	1.13	0.11	0.13	0.77	0.09	0.08	9.24***	
9								−0.33
Stage 6 Size 'x'	6.44	0.25		6.95	0.18			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								0.96
1–2	0.78	0.15	0.20	1.10	0.21	0.29	−3.12**	
2								−0.80
2–4	1.49	0.11	0.32	1.16	0.08	0.24	9.17***	
4								2.57*
4–6	0.93	0.07	0.15	0.66	0.07	0.11	11.19***	
6								5.90***
Stage 6 Size 'x'	7.06	0.24		6.60	0.30			

Table 21

'x' Distance 2–10 (mm). Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
3								8.45***
3–4	0.71	0.04	0.07	0.67	0.03	0.07	2.19*	
4								9.18***
4–6	2.43	0.08	0.23	2.33	0.07	0.24	3.14**	
6								9.18***
6–7	2.22	0.08	0.17	2.50	0.05	0.21	−11.85***	
7								4.36***
7–8	0.71	0.06	0.04	0.84	0.06	0.05	−6.30***	
8								3.22**
8–9	0.49	0.09	0.03	0.56	0.11	0.03	−1.80	
9								2.29*
Stage 6 Size 'x'	12.59	0.17		11.82	0.32			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
2								3.51***
2–3	2.48	0.08	0.39	2.66	0.06	0.43	−6.58***	
3								1.00
3–4	0.57	0.08	0.06	1.09	0.05	0.12	−20.38***	
4								−3.53**
4–6	1.67	0.06	0.17	1.96	0.07	0.20	−10.44***	
6								−5.98***
6–7	2.93	0.08	0.26	2.35	0.07	0.19	18.67***	
7								−2.15*
7–9	1.65	0.10	0.11	1.29	0.07	0.09	10.86***	
9								−0.47
Stage 6 Size 'x'	11.08	0.41		11.82	0.31			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
2								0.52
2–4	3.48	0.20	0.53	3.17	0.08	0.48	5.76***	
4								2.01
4–6	2.32	0.13	0.23	1.85	0.04	0.18	15.08***	
6								9.64***
Stage 6 Size 'x'	12.32	0.31		11.60	0.20			

Table 22

'x' Distance 2–11 (mm)	Stage no.	EXPERIMENT 2								EXPERIMENT 4								EXPERIMENT 7							
		Even			Odd			$H_0: \Delta x_e = \Delta x_o$	$H_0: x_e = x_o$	Even			Odd			$H_0: \Delta x_e = \Delta x_o$	$H_0: x_e = x_o$	Even			Odd			$H_0: \Delta x_e = \Delta x_o$	$H_0: x_e = x_e$
		Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t	Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t	Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t
Inter-stage Longitudinal INCREMENTS 'Δx'	1																								
	2																4.38***								−0.45
										0.90	0.06	0.18	1.17	0.05	0.24	−12.71***		2.02	0.31	0.40	1.78	0.20	0.35	2.55*	
	3								7.55***								0.57								
		1.00	0.03	0.16	0.94	0.03	0.16	4.93***		0.81	0.05	0.13	1.26	0.02	0.21	−31.22***									
	4								10.81***								−4.64***								0.78
		1.68	0.03	0.23	1.69	0.03	0.25	−0.63		1.07	0.06	0.15	1.37	0.04	0.19	−13.06***		1.60	0.23	0.22	1.12	0.04	0.16	8.81***	
	6								8.14***								−7.37***								11.16***
		2.29	0.04	0.25	2.49	0.05	0.29	−11.32***		3.01	0.08	0.38	2.50	0.06	0.29	16.15***									
	7								5.04***								−2.50*								
		0.81	0.04	0.07	0.86	0.03	0.07	−4.52***		1.37	0.09	0.12	1.11	0.03	0.10	10.19***									
	8								4.77***																
		0.30	0.05	0.02	0.27	0.03	0.02	1.72																	
	9								4.80***								−0.13								
Stage 6 Size 'x'		8.93	0.14		8.39	0.25				7.82	0.35		8.53	0.21				8.67	0.17		8.04	0.22			

Table 23

'x' Distance 3–4 (mm)	Stage no.	EXPERIMENT 2								EXPERIMENT 4								EXPERIMENT 7							
		Even			Odd			$H_0: \Delta x_e = \Delta x_o$	$H_0: x_e = x_o$	Even			Odd			$H_0: \Delta x_e = \Delta x_o$	$H_0: x_e = x_o$	Even			Odd			$H_0: \Delta x_e = \Delta x_o$	$H_0: x_e = x_e$
		Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t	Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t	Mean.	s.d.	Rel. Inc.	Mean.	s.d.	Rel. Inc.	t	t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								−1.09								−1.09								−1.09
		4.57	0.24	0.60	4.02	0.16	0.52	4.48***		1.94	0.26	0.25	1.77	0.14	0.22	1.50		2.28	0.21	0.29	2.52	0.12	0.32	−2.70*	
	2																1.77								−1.38
										1.80	0.08	0.18	2.22	0.05	0.23	−17.49***		2.97	0.10	0.29	2.53	0.09	0.25	12.14***	
	3								4.47***								−0.79								
		0.56	0.15	0.04	0.84	0.05	0.07	−6.36***		0.62	0.07	0.05	1.00	0.04	0.08	−17.93***									
	4								2.31*								−3.71***								3.59***
		0.94	0.08	0.07	0.88	0.05	0.06	2.19*		0.61	0.10	0.04	0.71	0.06	0.05	−3.07**		0.69	0.06	0.05	0.72	0.05	0.05	−1.38	
	6								5.24***								−5.89***								3.28**
		1.27	0.06	0.09	1.38	0.05	0.10	−5.50***		1.69	0.09	0.13	1.16	0.04	0.08	18.21***									
	7								0.46								−2.20*								
		0.39	0.03	0.02	0.31	0.04	0.02	6.18***		0.59	0.06	0.04	0.46	0.08	0.03	4.60***									
	8								3.90***																
		0.08	0.06	0.00	0.10	0.04	0.00	−1.20																	
	9								2.12*								−0.55								

Table 24

'x' Distance 3–7 (mm) — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								−1 24
	3 82	0 09	0 47	3 22	0 23	0 39	5 80***	
2								
3								7 38***
	0 90	0 06	0 07	0 85	0 03	0 07	2 12*	
4								8 01***
	1 29	0 03	0 10	1 60	0 05	0 13	−18 15***	
6								4 74***
	1 93	0 05	0 13	2 27	0 06	0 16	−16 18***	
7								−1 07
	0 58	0 04	0 03	0 61	0 05	0 03	−1 85	
8								−0 22
	0 17	0 04	0 01	0 21	0 04	0 01	−2 22*	
9								−0 49
Stage 6 Size 'x'	14 07	0 20		13 70	0 29			

EXPERIMENT 4

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								−1 24
	1 90	0 10	0 24	1 35	0 17	0 16	6 83***	
2								4 23***
	1 34	0 05	0 13	1 87	0 04	0 19	−31 57***	
3								−0 32
	0 64	0 04	0 05	1 15	0 03	0 10	−34 14***	
4								−6 50***
	0 97	0 07	0 08	1 04	0 02	0 08	−3 32**	
6								−6 98***
	2 67	0 10	0 20	2 14	0 05	0 15	16 58***	
7								−1 45
	0 89	0 05	0 05	0 85	0 04	0 05	2 00	
8								
9								−0 28
Stage 6 Size 'x'	12 84	0 37		13 51	0 17			

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Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_e$ t
1								−1 24
	2 15	0 11	0 27	2 03	0 13	0 25	1 97	
2								0 65
	2 61	0 07	0 25	2 02	0 07	0 20	22 75***	
3								
4								10 68***
	1 10	0 02	0 08	1 13	0 02	0 09	−3 95***	
6								9 33***
7								
8								
9								
Stage 6 Size 'x'	13 81	0 17		13 22	0 26			

Table 25

'x' Distance 3–9 (mm) — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								0 04
	1 05	0 09	0 30	0 96	0 28	0 28	0 70	
2								
3								1 66
	0 55	0 04	0 12	0 50	0 03	0 11	3 67**	
4								2 61*
	0 21	0 03	0 04	0 20	0 04	0 04	0 53	
6								2 89**
	0 12	0 03	0 02	0 11	0 03	0 02	1 03	
7								1 76
	0 13	0 04	0 02	0 15	0 02	0 02	−1 56	
8								2 72**
	−0 04	0 04	−0 00	−0 05	0 04	−0 01	0 66	
9								2 43*
Stage 6 Size 'x'	5 22	0 13		5 09	0 15			

EXPERIMENT 4

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								0 04
	−0 03	0 13	−0 00	−0 08	0 27	−0 02	0 39	
2								0 67
	0 86	0 05	0 24	0 92	0 05	0 26	−3 21**	
3								0 78
	0 31	0 03	0 07	0 51	0 03	0 11	−17 59***	
4								−3 01**
	0 22	0 05	0 04	0 23	0 02	0 04	−0 69	
6								−3 78***
	0 34	0 05	0 06	0 21	0 04	0 04	6 63***	
7								−1 39
	0 00	0 03	0 00	0 02	0 04	0 00	−1 82	
8								
9								−1 25
Stage 6 Size 'x'	4 98	0 16		5 16	0 11			

EXPERIMENT 7

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_e$ t
1								0 04
	0 21	0 13	0 06	0 43	0 18	0 12	−2 58*	
2								−2 45*
	1 32	0 06	0 35	1 02	0 05	0 26	13 62***	
3								
4								3 51***
	0 21	0 03	0 04	0 16	0 03	0 03	5 22***	
6								3 89***
7								
8								
9								
Stage 6 Size 'x'	5 24	0 18		5 04	0 17			

Table 26

'x' Distance 4–5 (mm) — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								−1.00
	1.71	0.12	0.64	0.95	0.14	0.32	9.83***	
2								
3								3.86***
	0.27	0.14	0.06	0.14	0.08	0.03	2.94**	
4								2.42*
	−0.09	0.10	−0.02	0.08	0.05	0.02	−5.64***	
6								0.60
	0.88	0.06	0.20	0.89	0.07	0.21	−0.31	
7								0.46
	0.13	0.05	0.02	0.14	0.05	0.02	−0.57	
8								1.13
	0.00	0.05	0.00	−0.02	0.05	−0.00	1.61	
9								0.09
Stage 6 Size 'x'	4.29	0.20		4.24	0.27			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								−1.00
	1.54	0.23	0.56	1.11	0.15	0.38	3.93**	
2								1.51
	0.11	0.08	0.02	0.31	0.07	0.08	−7.48***	
3								−0.5
	−0.00	0.11	−0.00	0.07	0.06	0.01	−2.58*	
4								−0.70
	−0.17	0.09	−0.04	−0.14	0.06	−0.03	−0.88	
6								−0.39
	1.02	0.08	0.24	0.92	0.06	0.22	3.06**	
7								0.43
	0.05	0.07	0.01	0.09	0.07	0.01	−1.38	
8								
9								0.28
Stage 6 Size 'x'	4.17	0.18		4.19	0.18			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								−1.00
	1.26	0.09	0.47	1.19	0.08	0.41	1.54	
2								0.92
	0.36	0.08	0.09	0.32	0.07	0.08	1.21	
3								
4								0.
	−0.04	0.07	−0.01	−0.11	0.05	−0.02	3.56**	
6								3.14**
Stage 6 Size 'x'	4.25	0.17		4.08	0.20			

Table 27

'x' Distance 4–7 (mm) — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								−1.43
	2.78	0.16	0.44	2.57	0.05	0.41	2.99**	
2								
3								1.58
	0.55	0.03	0.06	0.55	0.03	0.06	0.12	
4								2.76**
	0.41	0.03	0.04	0.45	0.03	0.04	−2.57*	
6								1.14
	−0.09	0.05	−0.00	−0.01	0.06	−0.00	−4.04***	
7								−0.56
	0.22	0.03	0.02	0.28	0.03	0.02	−5.81***	
8								−0.77
	0.13	0.04	0.01	0.16	0.03	0.01	−2.48*	
9								−0.81

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								−1.43
	1.24	0.05	0.20	1.14	0.04	0.18	3.60**	
2								0.68
	1.14	0.05	0.15	1.38	0.04	0.18	−14.12***	
3								−2.20*
	0.35	0.04	0.04	0.53	0.02	0.06	−13.57***	
4								−4.82***
	0.21	0.04	0.02	0.24	0.05	0.02	−1.35	
6								−4.95***
	0.46	0.09	0.05	0.24	0.03	0.02	8.30***	
7								−2.03
	0.19	0.04	0.01	0.19	0.03	0.02	−0.40	
8								
								−2.35*
9								

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								−1.43
	1.19	0.10	0.19	1.33	0.05	0.21	−3.22**	
2								−0.83
	1.84	0.06	0.24	1.54	0.06	0.20	12.46***	
3								
4								3.43**
	0.34	0.03	0.03	0.31	0.03	0.03	1.99	
6								3.39**

Table 28

'x' Distance 5–7 (mm) — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								0.53
	3.33	0.21	0.59	2.97	0.08	0.53	3.78**	
3								4.73***
	0.89	0.06	0.10	0.91	0.02	0.10	−1.34	
4								7.61***
	0.93	0.04	0.09	0.93	0.02	0.10	−0.45	
6								6.08***
	0.63	0.03	0.05	0.71	0.03	0.06	−7.43***	
7								7.07***
	0.25	0.02	0.02	0.32	0.05	0.02	−5.08***	
8								3.16**
	−0.05	0.03	−0.00	−0.06	0.05	−0.00	0.78	
9								3.89***
Stage 6 Size 'x'	10.74	0.21		10.34	0.20			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								0.53
	1.26	0.09	0.22	1.24	0.07	0.22	0.41	
2								1.63
	1.54	0.03	0.22	1.88	0.06	0.27	−17.57***	
3								−2.27*
	0.76	0.03	0.09	1.02	0.02	0.11	−21.85***	
4								−5.79***
	0.62	0.05	0.06	0.80	0.04	0.08	−9.59***	
6								−8.22***
	1.25	0.06	0.12	0.82	0.02	0.07	21.58***	
7								−3.80***
	0.26	0.04	0.02	0.18	0.04	0.01	5.43***	
9								−3.19**
Stage 6 Size 'x'	9.85	0.25		10.50	0.21			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								0.53
	1.29	0.12	0.22	1.57	0.08	0.28	−5.15***	
2								0.07
	2.79	0.04	0.39	2.44	0.04	0.34	21.35***	
4								6.77***
	0.79	0.03	0.08	0.64	0.03	0.06	14.30***	
6								8.13***
Stage 6 Size 'x'	10.68	0.23		10.17	0.20			

Table 29

'x' Distance 7–8 (mm) — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								−0.19
	1.70	0.15	0.64	1.49	0.12	0.57	2.49*	
3								5.73***
	0.67	0.09	0.15	0.51	0.03	0.12	6.08***	
4								8.38***
	1.05	0.05	0.20	1.15	0.04	0.25	−4.97***	
6								6.90***
	1.70	0.11	0.27	1.96	0.04	0.34	−9.04***	
7								1.27
	0.76	0.11	0.09	0.62	0.08	0.08	4.32***	
8								3.41**
	0.05	0.07	0.00	0.30	0.10	0.03	−7.79***	
9								0.97
Stage 6 Size 'x'	6.12	0.16		5.75	0.17			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								−0.19
	0.69	0.13	0.25	0.75	0.14	0.29	−0.75	
2								2.46*
	0.66	0.04	0.19	0.79	0.03	0.23	−9.77***	
3								0.90
	0.34	0.03	0.08	0.71	0.02	0.17	−37.12***	
4								−6.01***
	0.75	0.07	0.16	0.90	0.02	0.18	−7.31***	
6								−8.57***
	2.27	0.04	0.43	1.97	0.02	0.34	20.62***	
7								−4.31***
	0.86	0.08	0.11	0.74	0.07	0.09	4.07***	
9								−0.38
Stage 6 Size 'x'	5.24	0.18		5.74	0.15			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								−0.19
	0.95	0.19	0.36	0.74	0.10	0.27	2.69*	
2								0.24
	1.53	0.09	0.43	1.17	0.04	0.33	13.54***	
4								6.74***
	0.87	0.04	0.17	0.79	0.02	0.17	7.01***	
6								10.67***
Stage 6 Size 'x'	5.90	0.13		5.43	0.17			

Table 30

'x' Distance 7–14 (mm) / Inter stage Longitudinal INCREMENTS Δx'	Stage no	EXPERIMENT 2 Even Mean	s d	Rel Inc	Odd Mean	s d	Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t	EXPERIMENT 4 Even Mean	s d	Rel Inc	Odd Mean	s d	Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t	EXPERIMENT 7 Even Mean	s d	Rel Inc	Odd Mean	s d	Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_e$ t
	1								-1 61								-1 61								-1 61
		8 83	0 14	0 79	7 60	0 26	0 68	10 06***		4 28	0 22	0 39	3 34	0 23	0 29	7 35***		4 61	0 18	0 41	4 61	0 17	0 41	-0 07	
	2																4 90***								0 50
										3 12	0 09	0 20	4 00	0 10	0 27	-24 28***		7 44	0 09	0 47	6 14	0 07	0 39	42 25***	
	3								8 87***								0 76								
		3 20	0 09	0 16	2 75	0 08	0 14	13 80***		2 27	0 08	0 12	3 69	0 06	0 20	-53 34***									
	4								10 85***								-5 48***								6 95***
		5 32	0 08	0 22	5 53	0 08	0 26	-6 34***		3 65	0 18	0 17	4 37	0 05	0 19	-14 45***		4 45	0 03	0 19	3 78	0 10	0 17	26 66***	
	6								10 79***								-9 04***								11 52***
		7 87	0 09	0 27	8 86	0 16	0 33	-21 11***		10 46	0 24	0 42	8 75	0 14	0 32	22 11***									
	7								5 20***								-2 50*								
		2 11	0 10	0 05	2 27	0 09	0 06	-4 83***		3 03	0 08	0 08	2 65	0 06	0 07	14 00***									
	8								4 50***																
		0 58	0 06	0 01	0 55	0 06	0 01	1 45																	
	9								4 90***								-0 92								
Stage 6 Size x'		28 57	0 44	26 65	0 66					24 38	0 92		26 52	0 45				27 58	0 53		25 64	0 65			

Table 31

x' Distance 10–11 (mm) / Inter-stage Longitudinal INCREMENTS 'Δx'	Stage no	EXPERIMENT 2 Even Mean	s d	Rel Inc	Odd Mean	s d	Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t	EXPERIMENT 4 Even Mean	s d	Rel Inc	Odd Mean	s d	Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t	EXPERIMENT 7 Even Mean	s d	Rel Inc	Odd Mean	s d	Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_e$ t
	1																								
	2																0 29								1 80
										2 08	0 08	0 65	1 97	0 05	0 62	4 31***		1 93	0 08	0 52	1 91	0 14	0 53	0 70	
	3								2 14*								0 72								
		0 00	0 04	0 00	0 02	0 03	0 00	-1 38		0 18	0 07	0 03	0 24	0 04	0 04	-2 58*									
	4								3 56***								0 32								1 73
		1 60	0 04	0 28	1 58	0 03	0 29	1 41		1 33	0 05	0 24	1 42	0 05	0 26	-4 44***		1 57	0 05	0 28	1 49	0 03	0 27	5 62***	
	6								5 89***								-1 69								3 09**
		0 48	0 11	0 06	0 51	0 04	0 07	-1 32		0 61	0 07	0 09	0 49	0 04	0 07	5 09***									
	7								2 99**								0 29								
		0 16	0 09	0 02	0 24	0 06	0 03	-3 02**		0 46	0 08	0 06	0 67	0 06	0 09	-7 87***									
	8								3 24**																
		0 42	0 07	0 05	0 38	0 06	0 04	2 17*																	
	9								2 92**								-2 05*								

Table 32

'x' Distance 11–13 (mm)

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								
2								
3								6.45***
	1.60	0.02	0.25	1.40	0.03	0.24	16.17***	
4								11.36***
	2.14	0.05	0.26	2.05	0.05	0.28	4.45***	
6								9.73***
	3.03	0.10	0.30	3.53	0.08	0.38	−15.37***	
7								3.13**
	0.82	0.08	0.06	0.82	0.04	0.06	−0.12	
8								2.50*
	−0.04	0.05	−0.00	0.10	0.04	0.00	−8.44***	
9								2.21*
Stage 6 Size 'x'	10.10	0.19		9.28	0.32			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								
2								3.44**
	1.41	0.11	0.31	1.78	0.09	0.44	−9.81***	
3								1.37
	1.04	0.04	0.17	1.72	0.03	0.29	−48.80***	
4								−5.21***
	1.46	0.07	0.21	1.82	0.05	0.24	−14.44***	
6								−8.29***
	4.15	0.09	0.49	3.46	0.07	0.36	21.03***	
7								−2.67*
	1.08	0.09	0.08	0.62	0.07	0.04	14.53***	
8								
9								0.90
Stage 6 Size 'x'	8.44	0.39		9.39	0.28			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								
2								−1.21
	3.65	0.08	0.87	2.92	0.41	0.63	6.90***	
3								
4								2.97**
	1.74	0.03	0.22	1.39	0.10	0.18	14.27***	
6								8.20***
Stage 6 Size 'x'	9.68	0.33		8.99	0.24			

Inter-stage Longitudinal INCREMENTS 'Δx'

Table 33

'x' Distance 13–14 (mm)

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								
2								
3								3.44**
	0.24	0.02	0.18	0.20	0.01	0.16	5.44***	
4								5.99***
	0.51	0.01	0.32	0.51	0.01	0.36	−0.98	
6								7.47***
	0.78	0.02	0.37	0.81	0.01	0.42	−4.27***	
7								4.54***
	0.22	0.02	0.07	0.27	0.02	0.09	−5.60***	
8								1.39
	0.16	0.02	0.05	0.12	0.02	0.04	4.78***	
9								2.24*
Stage 6 Size 'x'	2.08	0.05		1.94	0.06			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								
2								0.59
	0.24	0.05	0.24	0.28	0.02	0.29	−3.03**	
3								−0.16
	0.22	0.03	0.18	0.25	0.01	0.19	−2.37*	
4								−1.00
	0.35	0.03	0.23	0.44	0.01	0.29	−9.59***	
6								−5.43***
	0.91	0.03	0.49	0.89	0.02	0.45	1.93	
7								−3.21**
	0.46	0.03	0.16	0.42	0.01	0.14	3.71**	
8								
9								−2.62*
Stage 6 Size 'x'	1.84	0.08		1.98	0.07			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								
2								−0.13
	0.61	0.02	0.59	0.75	0.22	0.73	−2.47*	
3								
4								−0.44
	0.45	0.01	0.27	0.20	0.19	0.11	5.78***	
6								6.20***
Stage 6 Size 'x'	2.08	0.06		1.92	0.10			

Inter-stage Longitudinal INCREMENTS 'Δx'

Table 34

EXPERIMENT 2

'x' Angle 6 3 1	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								0.58
		8.41	1.43		8.94	2.57		−0.44	
	2								
	3								3.32**
		4.08	0.20		4.74	0.63		−3.83***	
	4								3.82***
		3.78	0.28		4.59	0.44		−5.58***	
	6								3.79***
		4.00	0.21		4.40	0.36		−3.76***	
	7								3.10**
		1.39	0.21		1.14	0.20		3.56**	
	8								1.68
		−0.68	0.16		−0.97	0.12		6.14***	
	9								3.47**
Stage 6 Size 'x'		149.72	1.22		148.36	1.07			

EXPERIMENT 4

'x' Angle 6 3 1	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								0.58
		5.21	1.87		3.36	2.09		1.76	
	2								3.46**
		3 83	0.46		4.87	0.46		−6.31***	
	3								0.88
		3.64	0.37		5.66	0.40		−14.43***	
	4								−1.92
		3.24	0.44		3.82	0.19		−4.58**	
	6								−4.72***
		5.36	0.37		4.47	0.23		7.16***	
	7								−1.44
		0.66	0.18		0.40	0.31		2.59*	
	8								
	9								−1.83
Stage 6 Size 'x'		147.14	1.25		149.24	1.33			

EXPERIMENT 7

'x' Angle 6 3 1	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								0.58
		6.27	1.27		7.13	2.05		−0.96	
	2								2.83**
		7.26	0.40		6.77	0.43		−2.74*	
	3								
	4								3.43**
		2.83	0.25		2.39	0.35		4.44***	
	6								4.06***
	7								
	8								
	9								
Stage 6 Size 'x'		149.44	1.66		147.67	1.39			

Table 35

EXPERIMENT 2

'x' Angle 6 3/4 7	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								0.49
		4.83	2.02		6.59	1.58		−1.69	
	2								
	3								−1.51
		−0.71	1.04		0.98	0.63		−5.25***	
	4								−1.59
		0.67	0.62		−0.58	0.37		6.36***	
	6								1.14
		−3.46	0.33		−4.77	0.37		10.66***	
	7								5.16***
		−0.65	0.32		−1.13	0.40		3.98***	
	8								3.75***
		−0.63	0.32		−0.60	0.31		−0.34	
	9								3.33**

EXPERIMENT 4

'x' Angle 6 3/4 7	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								0.49
		−0.57	2.86		2.93	1.41		−2.87*	
	2								−1.23
		3.25	0.63		2.93	0.53		1.55	
	3								−0.64
		1.63	0.71		1.50	0 33		0.71	
	4								−0.56
		0.06	0.72		0.59	0 52		−2.24*	
	6					M			−3.07**
		−4.70	0.41		−5.43	0 27		5.62***	
	7								−1.28
		−1.35	0.25		−1.85	0.46		3.52**	
	8								
	9								−0.80

EXPERIMENT 7

'x' Angle 6 3/4 7	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								0.49
		0.60	1.03		4.77	1.46		−6.27***	
	2								−3.26**
		4.73	0.50		5.07	0.67		−1.62	
	3								
	4								−2.65*
		−0.43	0.49		−1.08	0.38		4.67***	
	6								−1.92
	7								
	8								
	9								

Table 36

'x' Angle 6 3 11 — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								
2								
3								2 63*
	2 71	0 30		2 11	0 64		3 28**	
4								4 06***
	6 76	0 38		8 33	0 51		-8 99***	
6								4 75***
	5 32	0 39	b	6 05	0 29		-6 25***	
7								2 66*
	1 42	0 44		1 41	0 23		0 07	
8								3 33**
	0 36	0 22		-0 10	0 17		7 39***	
9								4 52***
Stage 6 Size 'x'	104 92	1 50		102 79	1 36			

EXPERIMENT 4

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								
2								0 30
	0 52	1 04		0 45	0 70		0 22	
3								0 11
	3 16	0 47		4 37	0 33		-8 34***	
4								-1 30
	5 00	0 40		5 99	0 33		-6 96***	
6								-4 55***
	8 60	0 51		6 53	0 24		13 24***	
7								-0 43
	1 01	0 50		1 65	0 40		-3 75***	
8								
9								-2 01
Stage 6 Size 'x'	100 08	1 40		102 52	1 68			

EXPERIMENT 7

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_e$ t
1								
2								1 46
	2 30	0 76		5 23	3 78		-3 03***	
3								
4								2 70
	5 69	0 24		3 97	0 30		19 70***	
6								6 54***
7								
8								
9								
Stage 6 Size 'x'	104 21	1 91		101 13	1 32			

Table 37

x' Angle 5-6 8 — Inter-stage Longitudinal INCREMFNTS Δx

EXPERIMENT 2

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								1 10
	9 40	2 30		8 94	2 59		0 33	
2								
3								-1 58
	-1 06	0 71		0 53	1 02		-4 91***	
4								-4 52***
	-4 54	0 56		-8 07	0 84		12 61***	
6								-0 80
	-5 12	0 67		-5 65	0 59		2 45*	
7								0 29
	-2 40	0 29		-2 71	0 43		2 44*	
8								1 25
	-1 49	0 40		-1 31	0 47		-1 24	
9								-0 14
Stage 6 Size x	72 31	2 13		72 79	1 71			

EXPERIMENT 4

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								1 10
	5 63	0 33		10 37	1 49		-7 58***	
2								-2 88**
	6 14	1 25		4 09	1 04		5 03***	
3								-0 53
	-3 29	0 90		-5 97	0 59		10 01***	
4								3 27**
	-4 88	0 94		-5 05	0 56		0 57	
6								4 10***
	-4 52	0 90		-3 68	0 48		-2 96**	
7								1 74
	-3 62	0 54		-2 45	0 50		-6 00***	
8								
9								1 11
Stage 6 Size x	74 74	2 94		71 52	1 67			

EXPERIMENT 7

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_e$ t
1								1 10
	1 99	1 72		7 42	1 84		-5 87***	
2								-2 32*
	2 58	1 16		3 82	0 81		-3 49**	
3								
4								-5 33***
	-2 53	0 76		-3 41	0 66		3 86***	
6								-3 61***
7								
8								
9								
Stage 6 Size x	72 43	3 17		75 33	2 41			

Table 38

'x' Angle 5 6 3 — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								1.14
	10.75	2.11		10.04	1.85		0.61	
2								
3								−1.24
	−0.41	0.64		1.57	0.79		−7.44***	
4								−4.78***
	−4.14	0.49		−7.41	0.74		13.23***	
6								−0.44
	−4.06	0.67		−4.74	0.61		3.06**	
7								1.05
	−1.85	0.32		−2.35	0.46		3.75***	
8								2.23*
	−1.92	0.39		−1.88	0.46		−0.28	
9								0.96
Stage 6 Size 'x'	57.31	1.92		57.56	1.75			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								1.14
	7.10	0.78		10.50	1.41		−5.23***	
2								−2.32*
	6.57	1.08		4.86	0.95		4.74***	
3								−0.50
	−2.56	0.80		−4.74	0.48		9.37***	
4								2.97**
	−4.58	0.91		−4.57	0.56		−0.01	
6								3.71***
	−3.69	0.82		−2.85	0.45		−3.22**	
7								1.44
	−3.50	0.57		−2.48	0.49		−5.10***	
8								
9								0.56
Stage 6 Size 'x'	59.28	2.55		56.70	1.53			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								1.14
	3.49	1.63		8.99	1.47		−6.85***	
2								−1.57
	3.34	1.11		5.04	0.73		−5.06***	
3								
4								−5.27***
	−2.36	0.69		−3.22	0.56		4.31***	
6								−3.75***
7								
8								
9								
Stage 6 Size 'x'	57.43	2.57		59.89	2.00			

Table 39

'x' Angle 6 5 4 — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								−1.62
	−16.11	1.43		−13.20	4.12		−1.63	
2								
3								−1.68
	−4.89	1.06		−6.07	1.34		2.63*	
4								−0.28
	−3.46	0.74		−0.33	1.03		−8.96***	
6								−6.53***
	−3.38	0.77		−4.78	0.90		4.77***	
7								−3.01**
	1.31	0.52		1.01	0.77		1.33	
8								−2.52*
	3.43	0.56		3.79	0.79		−1.59	
9								−3.09**

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								−1.62
	−8.04	2.73		−10.40	2.75		1.55	
2								0.11
	−10.87	1.45		−10.95	1.29		0.16	
3								0.64
	−2.13	1.10		−1.08	0.62		−3.38**	
4								−1.92
	−2.37	0.82		−3.95	0.63		5.64***	
6								0.14
	−6.32	0.83		−4.98	0.53		−4.91***	
7								0.03
	1.24	0.85		1.24	0.58		−0.00	
8								
9								−0.24

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								−1.62
	−4.69	1.83		−9.61	2.42		4.38***	
2								−1.12
	−14.11	1.23		−14.58	0.90		1.22	
3								
4								0.59
	−5.42	0.76		−3.75	0.77		−6.88***	
6								−1.68
7								
8								
9								

Table 40

'x' Angle 5 4 3 — Inter stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_o $\Delta x_e = \Delta x_o$ t	H_o $x_e = x_o$ t
1								1 55
	5 93	0 80		4 74	3 00		0 94	
2								
3								6 11***
	6 51	0 44		5 94	0 40		3 57**	
4								7 63***
	11 10	0 41		12 35	0 55		-6 56***	
6								9 83***
	10 12	0 35		12 47	0 65		-12 46***	
7								3 64***
	0 98	0 42		1 69	0 46		-4 81***	
8								2 33*
	-1 83	0 38		-2 21	0 43		2 82**	
9								3 40**
Stage 6 Size 'x'	160 85	1 70		155 92	1 50			

EXPERIMENT 4

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_o $\Delta x_e = \Delta x_o$ t	H_o $x_e = x_o$ t
1								1 55
	0 31	2 50		-0 30	2 73		0 42	
2								4 20***
	4 83	0 33		7 60	0 98		-10 12***	
3								-0 16
	6 88	0 72		9 01	0 46		-9 94***	
4								-2 31*
	10 00	0 61		11 89	0 58		-8 10***	
6								-5 92***
	13 13	0 53		9 72	0 45		17 24***	
7								-1 91
	3 06	0 53		1 92	0 35		6 80***	
8								
9								-0 16
Stage 6 Size 'x'	155 60	1 93		159 71	2 10			

EXPERIMENT 7

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_o $\Delta x_e = \Delta x_o$ t	H_o $x_e = x_e$ t
1								1 55
	2 29	0 92		2 77	2 02		-0 58	
2								4 56***
	13 92	0 62		12 19	0 47		8 74***	
3								
4								5 56***
	10 31	0 35		9 15	0 45		8 96***	
6								8 24***
7								
8								
9								
Stage 6 Size 'x'	161 07	2 41		156 08	1 80			

Table 41

'x' Angle 6 5/7 8 — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_o $\Delta x_e = \Delta x_o$ t	H_o $x_e = x_o$ t
1								-0 43
	-17 12	1 77		-16 18	2 63		-0 72	
2								
3								0 24
	0 25	0 98		-2 30	1 26		6 12***	
4								3 50**
	5 92	0 68		10 63	1 00		-14 14***	
6								-0 74
	3 24	0 76		4 03	0 71		-3 12**	
7								-1 90
	3 07	0 43		3 32	0 53		-1 53	
8								-2 97**
	2 26	0 50		2 30	0 57		-0 21	
9								-1 44
Stage 6 Size x'	117 66	2 29		118 16	1 99			

EXPERIMENT 4

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_o $\Delta x_e = \Delta x_o$ t	H_o $x_e = x_o$ t
1								-0 43
	-9 36	1 44		-11 79	2 10		2 38*	
2								1 80
	-9 93	1 46		-9 29	1 09		-1 42	
3								0 65
	1 93	1 28		5 11	0 66		-8 98***	
4								-3 01**
	5 85	1 36		5 36	0 67		1 21	
6								-3 73***
	3 62	0 85		2 83	0 53		2 84**	
7								-1 31
	5 68	0 59		4 82	0 56		4 00***	
8								
9								-0 53
Stage 6 Size x'	115 22	3 32		118 58	1 97			

EXPERIMENT 7

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_o $\Delta x_e = \Delta x_o$ t	H_o $x_e = x_e$ t
1								-0 43
	-5 92	1 81		-10 10	2 18		3 98***	
2								1 22
	-7 44	1 32		-10 04	0 93		6 41***	
3								
4								4 89***
	3 82	0 76		4 80	0 80		-3 96***	
6								3 48***
7								
8								
9								
Stage 6 Size x'	117 77	3 48		114 70	2 66			

Table 42

'x' Angle 4 7 2 — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Rel. Inc.	Odd Mean.	Odd s.d.	Rel. Inc.	$H_o: \Delta x_e = \Delta x_o$ t	$H_o: x_e = x_o$ t
1								0.23
	6.98	1.68		7.52	1.27		-0.63	
2								
3								-1.96
	-1.94	0.86		-0.65	0.54		-4.77***	
4								-2.25*
	-1.10	0.66		-3.33	0.50		9.81***	
6								1.78
	-2.86	0.34		-3.85	0.30		8.88***	
7								4.72***
	-1.72	0.31		-1.79	0.37		0.59	
8								3.35**
	-0.37	0.46		-0.21	0.38		-1.13	
9								2.13*
Stage 6 Size 'x'	82.71	1.17		81.88	1.74			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Rel. Inc.	Odd Mean.	Odd s.d.	Rel. Inc.	$H_o: \Delta x_e = \Delta x_o$ t	$H_o: x_e = x_o$ t
1								0.23
	0.95	2.73		4.89	1.04		-3.54**	
2								-1.53
	2.96	0.50		2.09	0.40		5.38***	
3								0.11
	-0.45	0.63		-0.50	0.42		0.25	
4								-0.10
	-0.88	0.65		-1.09	0.50		0.96	
6								-0.36
	-4.51	0.53		-5.11	0.35		3.35**	
7								-0.71
	-1.69	0.34		-2.25	0.45		3.70**	
8								
9								-0.09
Stage 6 Size 'x'	83.08	1.44		83.27	1.53			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Rel. Inc.	Odd Mean.	Odd s.d.	Rel. Inc.	$H_o: \Delta x_e = \Delta x_o$ t	$H_o: x_e = x_e$ t
1								0.23
	1.08	1.27		5.30	1.17		-6.69***	
2								-5.19***
	5.11	1.47		3.94	0.69		2.85**	
3								
4								-1.31
	-2.42	0.97		-2.03	0.38		-1.65	
6								-4.19***
Stage 6 Size 'x'	82.72	1.58		84.53	1.45			

Table 43

'x' Angle 4 7 8 — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Rel. Inc.	Odd Mean.	Odd s.d.	Rel. Inc.	$H_o: \Delta x_e = \Delta x_o$ t	$H_o: x_e = x_o$ t
1								-0.07
	11.19	1.42		12.73	1.77		-1.66	
2								
3								0.03
	-0.54	0.86		1.72	0.60		-8.13***	
4								-1.88
	-1.10	0.62		-3.80	0.48		12.57***	
6								2.43*
	-2.63	0.42		-4.06	0.45		9.35***	
7								5.24***
	-1.87	0.37		-2.10	0.35		1.90	
8								4.59***
	-0.97	0.41		-1.01	0.33		0.35	
9								3.35**
Stage 6 Size 'x'	97.39	1.34		96.16	1.84			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Rel. Inc.	Odd Mean.	Odd s.d.	Rel. Inc.	$H_o: \Delta x_e = \Delta x_o$ t	$H_o: x_e = x_o$ t
1								-0.07
	1.68	2.44		4.23	2.44		-1.87	
2								-0.94
	6.61	0.58		7.36	0.71		-3.19**	
3								-0.90
	2.27	0.62		1.12	0.50		5.74***	
4								0.55
	-1.21	0.82		-0.19	0.66		-3.58**	
6								-1.06
	-4.64	0.58		-5.41	0.48		3.59**	
7								-1.14
	-3.52	0.44		-4.18	0.57		3.43**	
8								
9								-0.60
Stage 6 Size 'x'	98.12	1.73		98.71	1.51			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Rel. Inc.	Odd Mean.	Odd s.d.	Rel. Inc.	$H_o: \Delta x_e = \Delta x_o$ t	$H_o: x_e = x_e$ t
1								-0.07
	3.04	1.18		5.87	1.72		-3.66**	
2								-2.71**
	8.83	0.38		10.08	0.68		-6.31***	
3								
4								-3.43**
	-1.89	0.44		-2.66	0.48		5.25***	
6								-2.56*
Stage 6 Size 'x'	97.70	1.82		99.06	1.92			

Table 44

'x' Angle 4 7/6 1	Stage no	EXPERIMENT 2								EXPERIMENT 4								EXPERIMENT 7							
		Even			Odd			H_o $\Delta x_e = \Delta x_o$	H_o $x_e = x_o$	Even			Odd			H_o $\Delta x_e = \Delta x_o$	H_o $x_e = x_o$	Even			Odd			H_o $\Delta x_e = \Delta x_o$	H_o $x_e = x_e$
		Mean	s d	Rel Inc	Mean	s d	Rel Inc	t	t	Mean	s d	Rel Inc	Mean	s d	Rel Inc	t	t	Mean	s d	Rel Inc	Mean	s d	Rel Inc	t	t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								0 57								0 57								0 57
		3 48	1 68		5 49	1 38		−2 26*		−2 04	2 68		2 80	1 22		−4 31***		−0 89	1 03		3 20	1 13		−7 27***	
	2																−1 79								−4 09***
										2 82	0 45		2 16	0 42		4 16***		3 97	0 39		3 85	0 74		0 55	
	3								−2 70*								−1 04								
		−1 35	0 97		−0 05	0 57		−4 33***		0 91	0 62		0 26	0 34		3 65**									
	4								−2 29*								−0 05								−3 60***
		0 27	0 63		−1 23	0 39		7 49***		−0 24	0 65		0 12	0 48		−1 68		−0 60	0 40		−1 26	0 33		5 67***	
	6								0 58								−1 70								−3 23**
		−4 51	0 29		−5 67	0 33		10 53***		−5 54	0 39		−6 26	0 32		5 04***									
	7								4 16***								−0 72								
		−1 20	0 29		−1 49	0 39		2 45*		−1 46	0 22		−1 82	0 40		2 90**									
	8								2 97**																
		−0 20	0 32		−0 02	0 32		−1 67																	
	9								2 16*								−0 04								
Stage 6 Size 'x'		107 44	1 00		107 20	1 65				108 16	0 97		108 89	1 41				107 98	1 16		109 17	1 41			

Table 45

x' Angle 8 9 2	Stage no	EXPERIMENT 2								EXPERIMENT 4								EXPERIMENT 7							
		Even			Odd			H_o $\Delta x_e = \Delta x_o$	H_o $x_e = x_o$	Even			Odd			H_o $\Delta x_e = \Delta x_o$	H_o $x_e = x_o$	Even			Odd			H_o $\Delta x_e = \Delta x_o$	H_o $x_e = x_e$
		Mean	s d	Rel Inc	Mean	s d	Rel Inc	t	t	Mean	s d	Rel Inc	Mean	s d	Rel Inc	t	t	Mean	s d	Rel Inc	Mean	s d	Rel Inc	t	t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								−0 18								−0 18								−0 18
		−0 06	1 17		2 22	1 26		−3 24**		4 31	2 69		4 71	2 14		−0 29		0 80	3 68		0 28	3 11		0 30	
	2																1 37								1 05
										−6 50	1 18		−4 88	0 89		−4 39***		−5 46	1 60		−3 54	1 22		−3 81***	
	3								−0 02								−1 47								
		−2 91	0 98		−4 10	1 06		3 12**		−2 55	1 02		−3 48	0 75		2 94**									
	4								0 51								−0 48								−0 13
		3 33	1 03		2 48	0 92		2 24*		2 66	1 03		2 33	0 67		1 00		3 19	1 16		2 17	0 60		3 44**	
	6								1 27								−0 99								1 34
		2 74	0 74		3 10	0 60		−1 57		3 74	0 96		2 09	0 52		5 50***									
	7								0 62								0 07								
		−2 18	0 60		−0 84	0 34		−8 42***		0 78	0 77		−0 46	0 76		4 36									
									−1 55																
		1 35	0 82		2 03	0 95		−2 32*																	
	9								−1 18								0 09								
Stage 6 Size x'		166 67	2 01		165 86	2 07				163 34	2 52		164 05	1 70				163 87	2 35		163 07	1 79			

Table 46

'x' Angle 7 8 9 — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								0.85
1–3	−6.82	1.38		−5.53	1.59		−1.49	
2								
3								−3.01**
3–4	−0.49	0.47		−1.20	0.49		3.94***	
4								−1.59
4–6	−1.92	0.53		−1.19	0.38		−4.13***	
6								−2.55*
6–7	−2.11	0.36		−1.82	0.36		−2.34*	
7								−2.73**
7–8	0.97	0.40		0.75	0.21		2.10*	
8								−2.37*
8–9	0.29	0.33		−0.20	0.37		4.27***	
9								−1.45
Stage 6 Size 'x'	*165.83*	*1.24*		*166.85*	*1.31*			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								0.85
1–2	−1.76	0.72		0.53	2.38		−2.26*	
2								−1.80
2–3	−1.73	0.63		−4.94	0.91		11.14***	
3								1.85
3–4	−2.51	0.79		−0.62	0.71		−7.09***	
4								−0.38
4–6	−1.18	1.01		−2.65	0.47		4.97***	
6								2.28*
6–7	−2.30	0.48		−1.20	0.40		−6.20***	
7								0.82
7–9	2.41	0.40		3.09	0.42		−4.32***	
8								0.98
9								
Stage 6 Size 'x'	*167.17*	*1.54*		*166.15*	*1.12*			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								0.85
1–2	−1.63	0.98		1.64	2.81		−2.92**	
2								−2.40*
2–4	−5.08	0.71		−6.77	0.73		6.54***	
3								
4								−1.74
4–6	−0.68	0.52		−0.56	0.36		−0.84	
6								−2.54*
7								
8								
9								
Stage 6 Size 'x'	*166.76*	*1.61*		*167.90*	*1.57*			

Table 47

'x' Angle 7 8/1 9 — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								−0.20
1–3	−6.23	1.27		−7.58	2.34		1.24	
2								
3								−2.11*
3–4	−2.79	0.44		−4.10	0.53		7.19***	
4								−0.56
4–6	1.97	0.34		3.10	0.50		−6.73***	
6								−2.39*
6–7	−0.75	0.38		−0.08	0.45		−4.60***	
7								−2.62*
7–8	0.45	0.31		0.44	0.30		0.10	
8								−2.26*
8–9	1.48	0.21		1.91	0.36		−4.33***	
9								−3.45***

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								−0.20
1–2	−0.19	1.91		2.10	2.52		−1.82	
2								−1.40
2–3	−6.97	0.56		−8.06	0.78		4.40***	
3								−0.49
3–4	−2.52	0.53		−2.80	0.60		1.39	
4								−0.38
4–6	1.01	0.83		0.43	0.46		2.27*	
6								0.15
6–7	0.56	0.57		0.25	0.34		1.63	
7								1.03
7–9	2.78	0.36		3.04	0.32		−2.06	
8								1.17
9								

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								−0.20
1–2	−1.74	1.73		−2.66	2.29		0.86	
2								−0.80
2–4	−7.64	0.51		−8.98	0.78		5.72***	
3								
4								−0.29
4–6	1.90	0.33		1.99	0.41		−0.75	
6								−0.18
7								
8								
9								

Table 48

'x' Angle 7 8/1 2

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								0.60
	-13.86	1.28		-14.19	1.36		0.43	
2								
3								-3.07**
	-4.48	0.43		-5.51	0.53		5.73***	
4								-2.63*
	-0.06	0.32		0.67	0.48		-4.57***	
6								-3.80***
	-3.05	0.41		-3.05	0.46		0.01	
7								-2.68*
	0.42	0.34		0.34	0.28		0.81	
8								-2.06*
	1.67	0.22		2.05	0.34		-3.88***	
9								-3.24**
Stage 6 Size 'x'	30.57	1.48		32.19	1.24			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								0.60
	-5.78	1.96		-1.73	2.20		-3.47***	
2								-3.34**
	-8.91	0.65		-11.13	0.69		9.16***	
3								-0.49
	-3.92	0.48		-5.04	0.61		6.54***	
4								0.46
	-1.37	0.79		-1.47	0.42		0.43	
6								1.02
	-2.81	0.61		-2.52	0.33		-1.49	
7								0.45
	3.68	0.39		3.44	0.32		1.81	
8								
9								1.19
Stage 6 Size 'x'	31.32	1.52		30.82	1.32			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								0.60
	-6.55	1.52		-6.06	1.90		-0.54	
2								-1.51
	-14.33	1.71		-13.78	0.67		-1.19	
4								-1.44
	2.02	1.40		0.40	0.41		4.95***	
6								-0.79
Stage 6 Size 'x'	30.87	1.61		31.23	1.64			

Inter-stage Longitudinal INCREMENTS 'Δx'

Table 49

'x' Angle 7 8/6 1

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								-0.57
	7.71	1.25		7.23	1.80		0.53	
2								
3								2.99**
	0.80	0.39		1.77	0.46		-5.98***	
4								0.81
	-1.37	0.25		-2.56	0.32		10.52***	
6								3.61***
	1.87	0.33		1.61	0.30		2.42*	
7								2.97**
	-0.66	0.25		-0.61	0.25		-0.69	
8								2.73**
	-0.77	0.17		-0.99	0.27		2.82**	
9								3.04**
Stage 6 Size 'x'	169.87	0.93		168.88	0.79			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								-0.57
	3.73	1.28		1.42	1.97		2.44*	
2								1.40
	3.79	0.48		5.20	0.55		-7.50***	
3								-0.20
	1.36	0.51		0.85	0.44		2.97**	
4								0.78
	-0.96	0.72		-0.31	0.36		-3.05**	
6								0.37
	0.89	0.40		0.85	0.28		0.31	
7								-0.78
	-2.05	0.26		-2.36	0.22		3.36**	
8								
9								-0.86
Stage 6 Size 'x'	169.88	1.26		169.74	0.92			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								-0.57
	3.93	0.91		2.67	1.89		1.60	
2								1.38
	4.86	0.33		6.22	0.49		-9.08***	
4								-0.55
	-1.29	0.24		-1.39	0.29		1.18	
6								-0.54
Stage 6 Size 'x'	169.64	1.09		169.82	1.20			

Inter-stage Longitudinal INCREMENTS 'Δx'

Table 50 — 'x' Angle 7 8/6 2 — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								-9.67
	6.19	1.28		6.11	1.54		0.10	
2								
3								1.79
	-0.03	0.25		0.22	0.48		-1.80	
4								0.53
	0.21	0.26		0.08	0.38		1.02	
6								2.43*
	-1.49	0.33		-0.05	0.33		-12.53***	
7								-2.30*
	0.26	0.28		-0.00	0.17		3.55**	
8								-1.36
	0.08	0.18		0.07	0.22		0.23	
9								-1.40
Stage 6 Size 'x'	179.22	0.55		178.75	0.67			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								-0.67
	3.21	1.31		1.28	1.59		2.35*	
2								0.82
	3.33	0.48		3.98	0.32		-4.48***	
3								0.75
	-0.07	0.53		0.49	0.35		-3.66**	
4								-0.63
	0.51	0.51		0.39	0.29		0.75	
6								-1.31
	-0.62	0.19		-0.50	0.36		-1.03	
7								0.70
	0.26	0.31		0.67	0.27		M-3.72**	
8								-0.21
9								
Stage 6 Size 'x'	178.89	0.76		179.21	0.66			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								-0.67
	4.00	0.93		2.01	1.69		2.76*	
2								1.25
	2.34	0.99		3.85	0.31		-5.76***	
3								
4								-0.35
	0.46	0.58		0.42	0.31		0.26	
6								-1.08
Stage 6 Size 'x'	178.91	0.63		179.11	0.67			

Table 51 — 'x' Angle 7 8/14 10 — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x$ t	$H_0: x_e = x_o$ t
1								
2								
3								-2.39*
	-2.78	0.48		-3.35	0.53		3.04**	
4								-2.52*
	-0.51	0.32		0.15	0.39		-4.80***	
6								-4.68***
	-1.57	0.39		-1.62	0.39		0.40	
7								-2.84**
	0.16	0.34		0.34	0.24		-1.91	
8								-2.79**
	1.15	0.20		1.18	0.27		-0.42	
9								-3.52**

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								
2								-1.59
	-8.11	0.47		-9.47	0.78		5.71***	
3								-0.43
	-3.36	0.44		-4.21	0.53		4.87***	
4								0.24
	0.01	0.65		-0.38	0.38		2.00	
6								0.80
	-1.09	0.51		-0.76	0.38		-1.81	
7								0.92
	2.12	0.33		2.52	0.18		-3.99***	
8								
9								1.16

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
2								-1.68
	-11.13	0.60		-12.00	0.53		4.30***	
3								
4								-1.79
	0.79	0.28		1.22	0.42		-3.75***	
6								-2.57*

Table 52

'x' Angle 1–2–5 — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								-0.23
1–3	6.43	1.44		7.36	0.83		-1.36	
3								0.90
3–4	3.83	0.29		3.88	0.40		-0.34	
4								2.30*
4–6	-0.03	0.22		-0.27	0.28		2.45*	
6								2.35*
6–7	-0.08	0.23		0.10	0.28		-1.99	
7								1.49
7–8	-0.30	0.19		-0.04	0.20		-4.02***	
8								0.17
8–9	-1.50	0.19		-1.73	0.25		3.03**	
9								1.72
Stage 6 Size 'x'	159.12	1.14		158.36	0.91			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
1								-0.23
1–2	1.02	1.93		-1.11	1.23		2.42*	
2								2.59*
2–3	6.74	0.60		7.31	0.41		-3.19**	
3								0.84
3–4	2.21	0.41		3.89	0.38		-11.94***	
4								-1.87
4–6	1.85	0.33		0.79	0.16		10.66***	
6								-0.74
6–7	0.11	0.46		0.38	0.24		-1.90	
7								-0.42
7–9	-2.67	0.32		-2.14	0.23		-5.09***	
9								-1.52
Stage 6 Size 'x'	158.56	1.04		158.84	1.15			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
1								-0.23
1–2	0.75	1.26		1.61	0.97		-1.50	
2								1.19
2–4	8.83	0.35		8.98	0.49		-0.97	
4								2.79**
4–6	-0.13	0.26		0.52	0.27		-7.87***	
6								0.41
Stage 6 Size 'x'	159.01	1.18		158.89	0.97			

Table 53

'x' Angle 1–2–11 — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
3								0.79
3–4	1.99	0.80		2.37	0.61		-1.44	
4								0.49
4–6	-2.60	0.86		-4.35	0.81		5.41***	
6								2.59*
6–7	1.44	0.56		1.73	0.33		-1.86	
7								0.63
7–8	-0.99	0.42		-0.62	0.37		-2.81***	
8								-0.82
8–9	-0.91	0.72		-0.29	0.99		-2.14*	
9								-0.16
Stage 6 Size 'x'	Δ 78.97	1.31		77.89	1.36			

EXPERIMENT 4

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
2								1.89
2–3	5.31	1.41		6.20	1.07		-2.02	
3								1.86
3–4	-1.02	0.57		1.44	0.58		-12.03***	
4								-2.10*
4–6	-0.88	0.54		-1.83	0.54		4.51***	
6								-0.43
6–7	0.40	0.82		0.48	0.50		-0.32	
7								-1.14
7–9	0.40	0.75		-1.55	0.53		8.06***	
9								-0.02
Stage 6 Size 'x'	78.19	1.99		78.51	2.33			

EXPERIMENT 7

Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
2								-1.46
2–4	13.04	3.79		6.52	3.12		5.30***	
4								1.04
4–6	-4.75	2.94		-1.70	0.35		-4.60***	
6								-1.20
Stage 6 Size 'x'	78.70	1.65		79.20	1.18			

Table 54

'x' Area 2 3 4 5 6 7 8 9 2 (mm^2) — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								−1 48
	68 50	2 07	1 41	57 85	1 91	1 16	9 23***	
2								
3								7 65***
	20 70	0 67	0 17	18 51	0 71	0 17	8 49***	
4								8 39***
	24 84	0 77	0 18	26 22	0 88	0 21	−4 29***	
6								6 75***
	35 52	0 80	0 22	39 44	1 27	0 26	−10 40***	
7								3 54***
	12 14	1 09	0 06	13 93	0 60	0 07	−6 29***	
8								3 30**
	3 81	0 81	0 01	3 94	0 83	0 01	−0 50	
9								2 80**
Stage 6 Size 'x'	161 64	4 32		150 88	5 74			

EXPERIMENT 4

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								−1 48
	27 12	1 32	0 56	22 95	1 21	0 46	5 91***	
2								2 89**
	28 31	0 78	0 36	35 52	0 78	0 49	−25 81***	
3								−1 01
	14 03	1 00	0 13	23 21	0 61	0 21	−31 65***	
4								−6 96***
	15 61	0 98	0 13	19 39	0 81	0 14	−10 95***	
6								−8 53***
	51 01	2 12	0 37	40 33	0 98	0 26	16 73***	
7								−3 48**
	15 11	0 88	0 08	12 55	0 91	0 06	7 66***	
8								
9								−2 52*
Stage 6 Size 'x'	134 75	6 69		150 13	3 92			

EXPERIMENT 7

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_e$ t
1								−1 48
	28 44	1 27	0 48	30 57	1 49	0 61	−2 95**	
2								−0 45
	57 26	1 27	0 73	46 09	0 84	0 58	29 19***	
3								
4								7 65***
	21 16	0 82	0 15	17 72	0 72	0 14	13 97***	
6								8 44***
Stage 6 Size 'x'	157 06	5 52		143 58	5 77			

Table 55

'x' Area 2 3 9 2 (mm^2) — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								0 78
	5 70	0 23	1 35	5 31	0 45	1 28	1 88	
2								
3								5 78***
	2 98	0 27	0 29	2 21	0 15	0 24	9 07***	
4								5 80***
	4 24	0 29	0 32	3 84	0 25	0 33	3 71**	
6								7 76***
	5 96	0 29	0 34	6 30	0 23	0 41	−3 85***	
7								3 24**
	1 62	0 37	0 07	2 22	0 24	0 10	−5 87***	
8								3 80***
	0 74	0 48	0 02	0 53	0 42	0 02	1 39	
9								3 07**

EXPERIMENT 4

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								0 78
	1 60	0 51	0 35	1 67	0 45	0 42	−0 27	
2								1 75
	2 77	0 21	0 44	3 01	0 18	0 52	−3 37**	
3								1 38
	1 47	0 13	0 16	2 89	0 11	0 33	−32 32***	
4								−4 45***
	2 75	0 29	0 26	3 42	0 20	0 29	−6 88***	
6								−6 07***
	8 25	0 45	0 62	6 60	0 31	0 42	10 80***	
7								−2 27*
	3 45	0 43	0 16	2 54	0 34	0 11	6 27***	
	3 45	0 43	0 16	2 54	0 34	0 11	6 27***	
8								
9								−1 02

EXPERIMENT 7

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_e$ t
1								0 78
	1 86	0 32	0 43	2 85	0 38	0 69	−5 33***	
2								−2 12*
	6 44	0 16	1 02	4 86	0 16	0 73	27 06***	
3								
4								4 87***
	3 80	0 19	0 29	2 57	0 21	0 21	18 95***	
6								7 06***

Table 56

'x' Area 2 9 14 1 2 (mm^2) — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								0 03
	23 15	0 82	1 58	19 11	1 51	1 35	5 75***	
2								
3								7 18***
	13 32	0 73	0 35	11 71	0 46	0 35	6 94***	
4								7 53***
	25 57	0 61	0 49	24 38	0 70	0 55	4 65***	
6								8 83***
	46 31	0 89	0 59	48 80	0 92	0 71	−7 88***	
7								5 16***
	18 39	0 89	0 13	18 68	0 64	0 15	−9 04***	
8								3 49**
	−0 66	6 27	−0 00	2 64	1 30	0 01	−2 30*	
9								0 41
Stage 6 Size 'x'	77 62	2 26		68 58	4 00			

EXPERIMENT 4

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								0 03
	8 19	0 65	0 56	5 29	0 88	0 36	6 64***	
2								4 54***
	10 59	0 41	0 46	13 17	0 42	0 65	−17 19***	
3								0 15
	8 86	0 63	0 26	13 52	0 25	0 40	−27 86***	
4								−4 97***
	16 00	0 81	0 37	22 25	0 54	0 47	−23 82***	
6								−8 88***
	59 78	1 15	1 02	51 33	1 33	0 74	16 63***	
7								−2 15*
	23 34	1 35	0 20	20 39	0 84	0 17	7 12	
8								
9								−0 31
Stage 6 Size 'x'	57 61	5 10		69 34	2 59			

EXPERIMENT 7

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_e$ t
1								0 03
	10 20	0 85	0 72	10 27	0 76	0 70	−0 16	
2								0 24
	25 47	2 54	0 99	20 89	0 50	0 85	5 52***	
3								
4								1 94
	23 54	1 77	0 47	17 14	0 64	0 36	15 12***	
6								10 39***
Stage 6 Size 'x'	73 31	3 03		63 12	3 88			

Table 57

'x' Area 7 5 9 14 10 7 (mm^2) — Inter-stage Longitudinal INCREMENTS 'Δx'

EXPERIMENT 2

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								
2								
3								8 24***
	7 33	0 46	0 28	5 08	0 32	0 23	15 01***	
4								11 38***
	26 91	0 81	0 79	25 66	0 85	0 98	3 88***	
6								9 68***
	42 79	1 01	0 70	47 76	1 18	0 92	−12 86***	
7								3 45**
	14 35	1 08	0 13	16 36	1 00	0 16	−5 83***	
8								1 72
	6 11	0 85	0 05	7 07	1 39	0 06	−2 48*	
9								1 34
Stage 6 Size 'x'	60 77	2 73		51 48	3 36			

EXPERIMENT 4

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_o$ t
1								
2								5 30***
	6 22	0 48	0 39	8 12	0 36	0 60	−12 65***	
3								−0 13
	5 05	0 88	0 23	10 39	0 54	0 48	−20 81***	
4								−4 77***
	14 63	1 19	0 55	17 80	0 73	0 56	−8 50***	
6								−6 99***
	53 65	1 22	1 29	44 82	0 79	0 88	21 71***	
7								−0 51
	22 60	1 00	0 24	20 18	1 23	0 21	5 76***	
8								
9								0 33
Stage 6 Size 'x'	40 70	4 77		50 11	3 18			

EXPERIMENT 7

Stage no	Even Mean	Even s d	Even Rel Inc	Odd Mean	Odd s d	Odd Rel Inc	H_0 $\Delta x_e = \Delta x_o$ t	H_0 $x_e = x_e$ t
2								2 32*
	20 32	0 59	1 39	15 62	0 35	1 13	27 22***	
3								
4								8 10***
	21 22	0 63	0 60	15 73	0 60	0 52	28 04***	
6								11 45***
Stage 6 Size 'x'	56 21	3 48		45 60	3 03			

Table 58

EXPERIMENT 2

'x' Area 1 2 9 8 7 10 14 1 (mm^2)	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								
	2								
	3								8.20***
		20.65	0.85	0.32	16.80	0.62	0.30	13.83***	
	4								10.49***
		52.49	0.92	0.61	50.05	1.32	0.71	5.51***	
	6								10.18***
		89.11	1.34	0.64	96.56	1.91	0.80	-12.77***	
	7								5.05***
		30.74	1.62	0.13	35.05	1.38	0.16	-8.71***	
	8								2.97**
		3.67	8.40	0.01	9.72	1.45	0.03	-3.17**	
	9								0.44
Stage 6 Size 'x'		138.40	4.09		120.07	6.98			

EXPERIMENT 4

'x' Area 1 2 9 8 7 10 14 1 (mm^2)	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								
	2								5.77***
		16.82	0.64	0.43	21.30	0.65	0.63	-19.24***	
	3								0.02
		13.92	1.13	0.25	23.91	0.60	0.43	-31.50***	
	4								-5.62***
		30.64	1.77	0.44	40.06	0.87	0.51	-18.08***	
	6								-8.58***
		113.44	2.18	1.13	96.16	1.81	0.80	21.59***	
	7								-1.62
		45.95	2.05	0.21	40.57	1.53	0.18	8.01***	
	8								
	9								-0.01
Stage 6 Size 'x'		98.32	9.81		119.45	4.39			

EXPERIMENT 7

'x' Area 1 2 9 8 7 10 14 1 (mm^2)	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								
	2								1.26
		44.80	2.70	1.14	36.52	0.62	0.95	11.91***	
	3								
	4								3.77***
		44.77	1.53	0.52	32.88	1.05	0.42	28.52***	
	6								12.00***
	7								
	8								
	9								
Stage 6 Size 'x'		129.53	5.85		108.72	6.40			

Table 59

EXPERIMENT 2

'x' Radius 12. 14.15 (mm)	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								
	2								
	3								3.94***
		0.72	0.02	0.21	0.63	0.02	0.20	8.38***	
									5.83***
		1.15	0.02	0.28	1.22	0.02	0.32	-6.92***	
	6								7.21***
		1.64	0.03	0.31	1.76	0.02	0.35	-11.20***	
	7								2.98**
		0.47	0.02	0.06	0.47	0.02	0.06	0.11	
	8								2.84**
		0.15	0.02	0.02	0.17	0.00	0.02	-4.72***	
	9								3.09**

EXPERIMENT 4

'x' Radius 12. 14.15 (mm)	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_o$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								
	2								1.48
		0.71	0.08	0.27	0.64	0.03	0.26	2.84**	
	3								3.52**
		0.50	0.07	0.15	0.83	0.02	0.27	-17.81***	
									-1.66
		0.80	0.07	0.21	1.05	0.04	0.26	-11.19***	
	6								-7.67***
		2.10	0.04	0.45	1.75	0.03	0.34	21.12***	
	7								-1.74
		0.73	0.03	0.11	0.55	0.01	0.08	6.17***	
	8								
	9								0.91

EXPERIMENT 7

'x' Radius 12. 14.15 (mm)	Stage no.	Even Mean.	Even s.d.	Even Rel. Inc.	Odd Mean.	Odd s.d.	Odd Rel. Inc.	$H_0: \Delta x_e = \Delta x_o$ t	$H_0: x_e = x_e$ t
Inter-stage Longitudinal INCREMENTS 'Δx'	1								
	2								-1.53
		1.59	0.04	0.61	1.41	0.04	0.53	11.59***	
	3								
									2.49*
		0.86	0.01	0.20	0.66	0.03	0.16	21.57***	
	6								7.94***
	7								
	8								
	9								

Table 60 TWO 'SHAPE INDICES' FOR THE CRANIA OF RATS FROM EXPERIMENTS 2 AND 4

Ratio: 2 5/(2 3 + 3 4 + 4 5)

		Even			Odd			Inter-group Differences	
		Mean	s.d.	N	Mean	s.d.	N	't'-value	
Exp. 2	Stage 3	0.9580	0.0053	22	0.9486	0.0054	20	5.69	* * *
	4	0.9727	0.0051	20	0.9639	0.0033	22	6.71	* * *
	6	0.9909	0.0018	20	0.9854	0.0018	21	9.84	* * *
	7	0.9982	0.0009	20	0.9968	0.0013	24	4.22	* * *
	8	0.9982	0.0008	20	0.9977	0.0041	23	1.72	(*)
	9	0.9978	0.0011	22	0.9967	0.0013	22	3.08	* *
Exp. 4	Stage 2	0.9355	0.0083	22	0.9283	0.0041	23	3.73	* * *
	3	0.9506	0.0068	18	0.9501	0.0091	20	0.19	N.S.
	4	0.9662	0.0036	16	0.9694	0.0040	19	—2.47	*
	6	0.9830	0.0033	15	0.9893	0.0022	20	—6.83	* * *
	7	0.9968	0.0011	16	0.9975	0.0009	21	—2.22	*
	9	0.9981	0.0009	18	0.9981	0.0005	22	—0.18	N.S.
Ratio: 2 5/4 7									
Exp. 2	Stage 3	2.2839	0.0616	22	2.1836	0.0421	20	6.10	* * *
	4	2.3426	0.0674	20	2.2482	0.0470	22	5.30	* * *
	6	2.5392	0.0523	20	2.4224	0.0446	21	7.71	* * *
	7	3.0538	0.1284	20	2.9502	0.1089	24	2.90	* *
	8	3.0763	0.1276	20	2.9746	0.1191	23	2.70	*
	9	3.0699	0.1794	22	2.9753	0.1513	22	1.89	(*)
Exp. 4	Stage 2	2.1920	0.0438	22	2.1284	0.0434	23	4.89	* * *
	3	2.2148	0.0464	18	2.1831	0.0573	20	1.86	(*)
	4	2.2735	0.0562	16	2.2932	0.0545	19	—1.05	N.S.
	6	2.4153	0.0487	15	2.4678	0.0699	20	—2.49	*
	7	2.9064	0.0918	16	2.9060	0.0840	21	+0.01	N.S.
	9	0.0576	0.1105	18	3.0129	0.1033	22	+1.32	N.S.

$p > 0.10$	N.S.
$0.05 < p < 0.10$	(*)
$0.01 < p < 0.05$	*
$0.001 < p < 0.01$	* *
$p < 0.001$	* * *

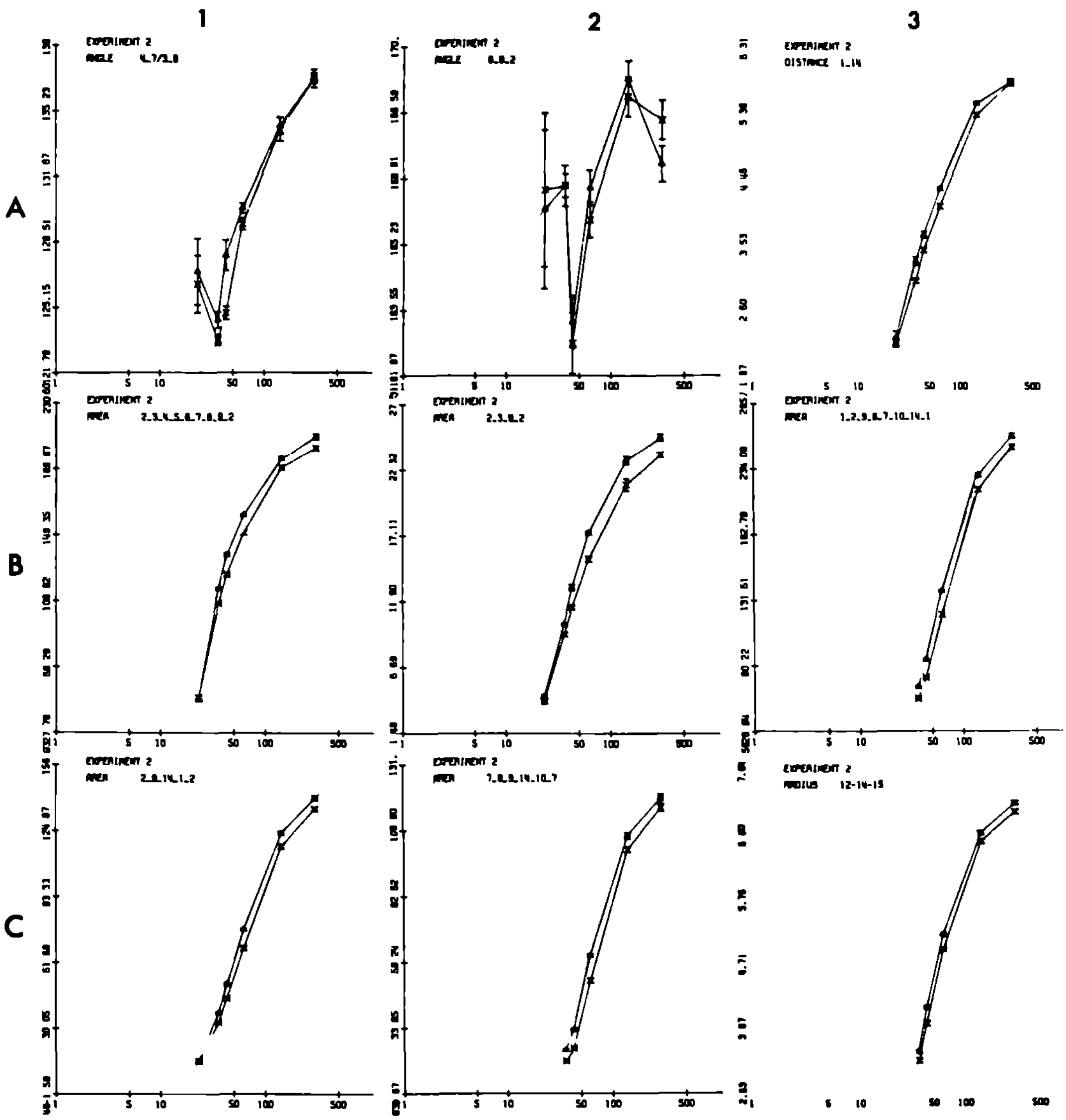
PLOTS OF MEAN QUANTITIES FROM EXPERIMENTS 2 AND 4

Comparable quantities are placed in corresponding locations on adjacent pages. They are located from the text by coordinates of numbers and letters found here.

In all cases in these graphs, an 'x' is the symbol for the odd-numbered or initially large-litter group.

The dimensions of distances are given in millimetres, and angles are given in degrees. Areas are in square millimetres. Time is scaled logarithmically for convenience only.

In both series the first registration is stage 1 (day 23) except in quantities involving points



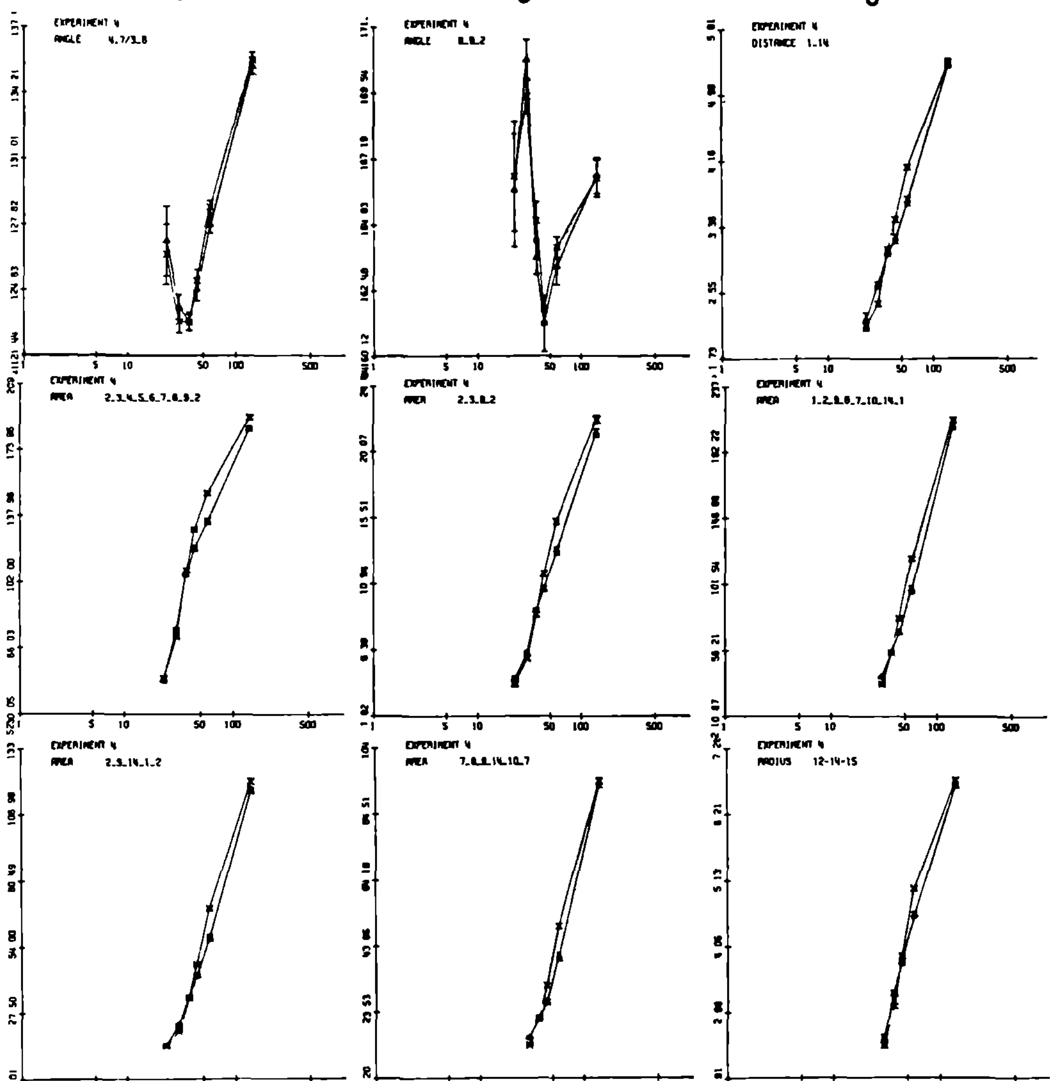
10, 11, 12, 13, 15, which do not exist in that stage. In these cases, experiment 2 graphs begin with stage 3 and experiment 4 graphs with stage 2.

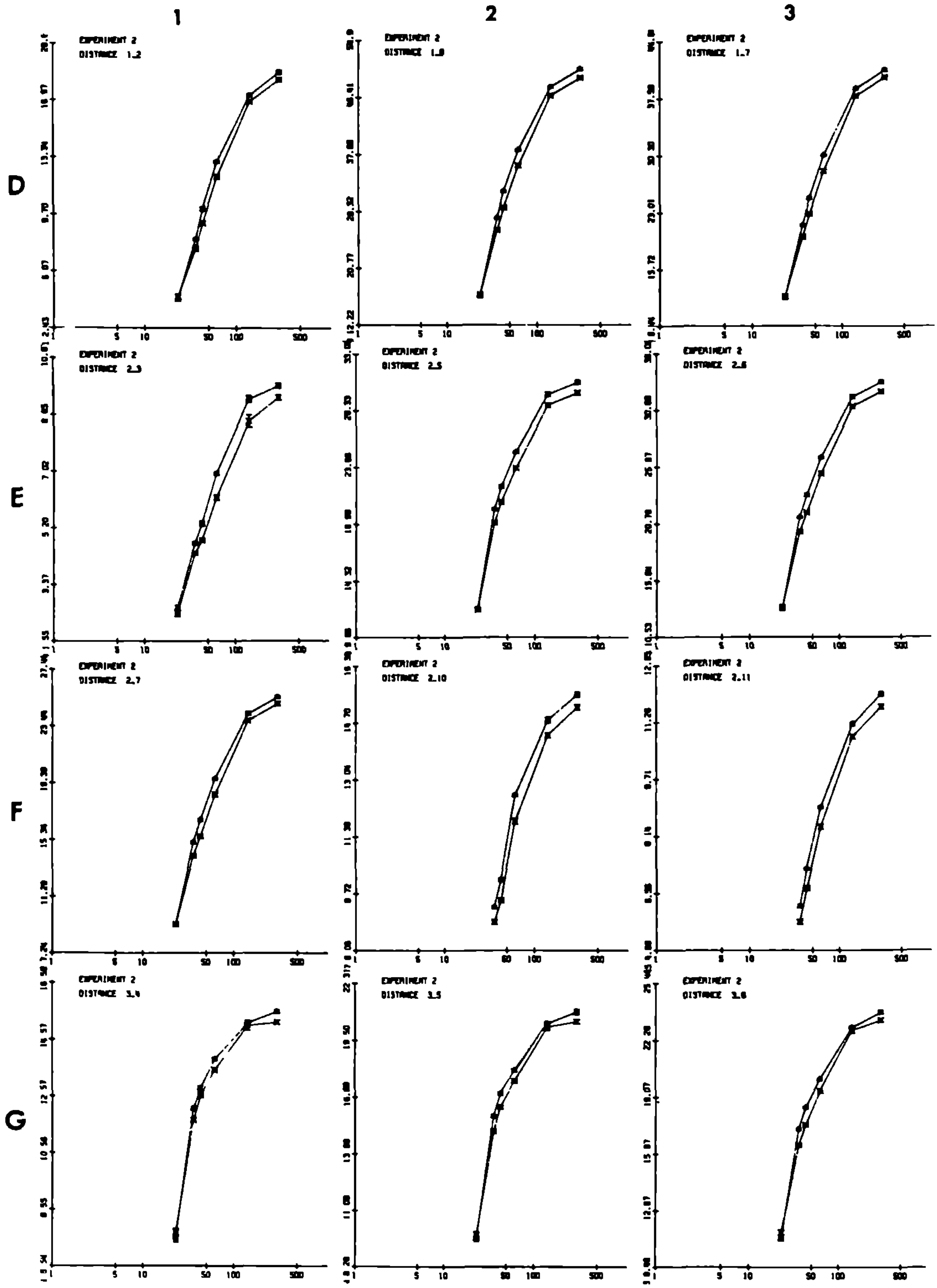
In experiment 2 the last stage graphed is stage 8, and in experiment 4 it is stage 7.

The graphs are plotted in such a way that the length of the graphs is always similar, and this requires that the vertical scale varies in most cases.

At each point registered, one standard error of the mean is plotted above and below the value being recorded. If the separation of the curves is greater than twice the sum of the standard errors, a difference of significance at better than a 5 % level of confidence exists.

4 5 6

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3
D
E
F
G
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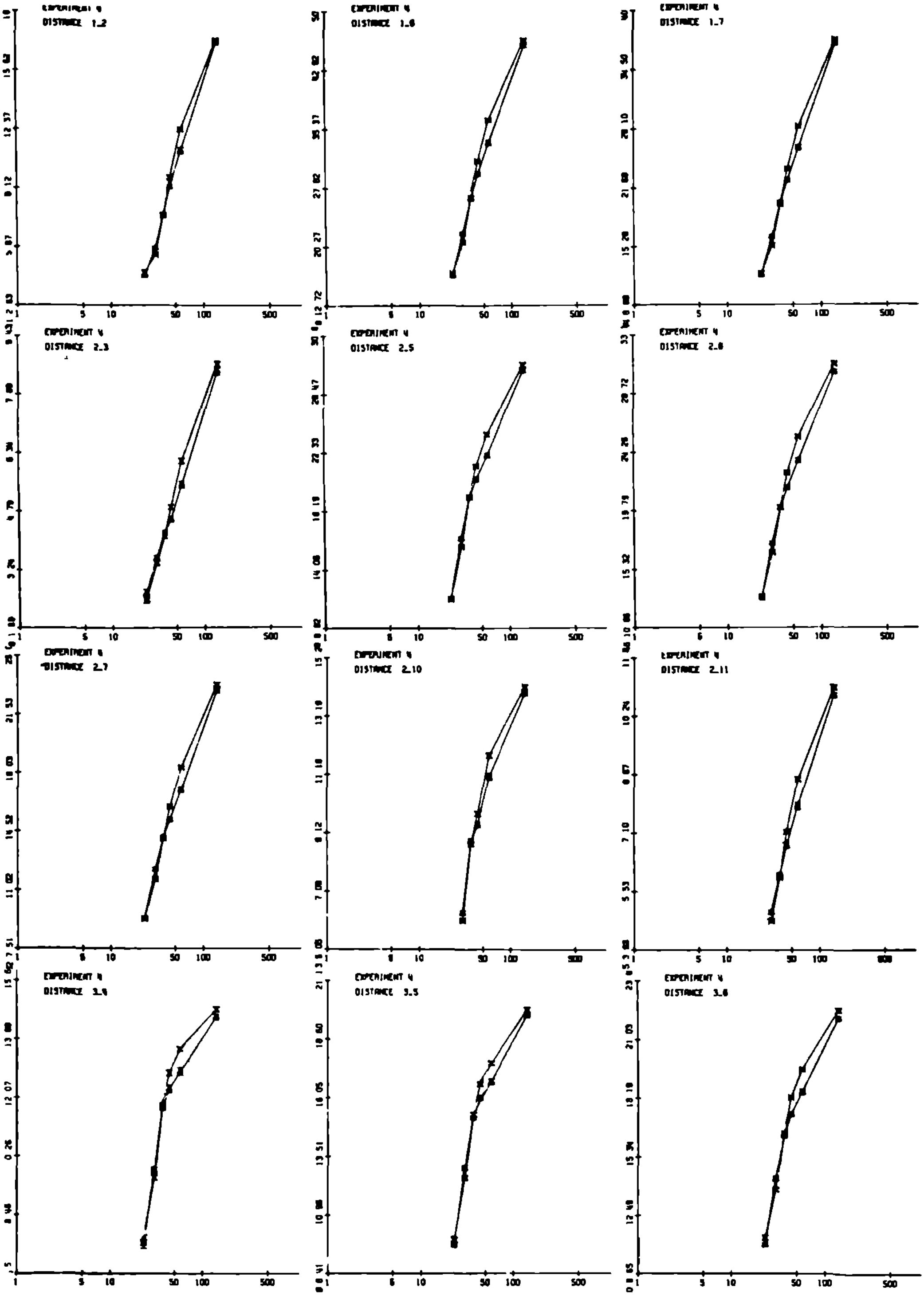
DISTANCE 1.8
DISTANCE 1.7
DISTANCE 2.3
DISTANCE 2.5
DISTANCE 2.6
DISTANCE 2.7
DISTANCE 2.10
DISTANCE 2.11
DISTANCE 3.4
DISTANCE 3.5
DISTANCE 3.6
1
5
10
50
100
500

4 5 6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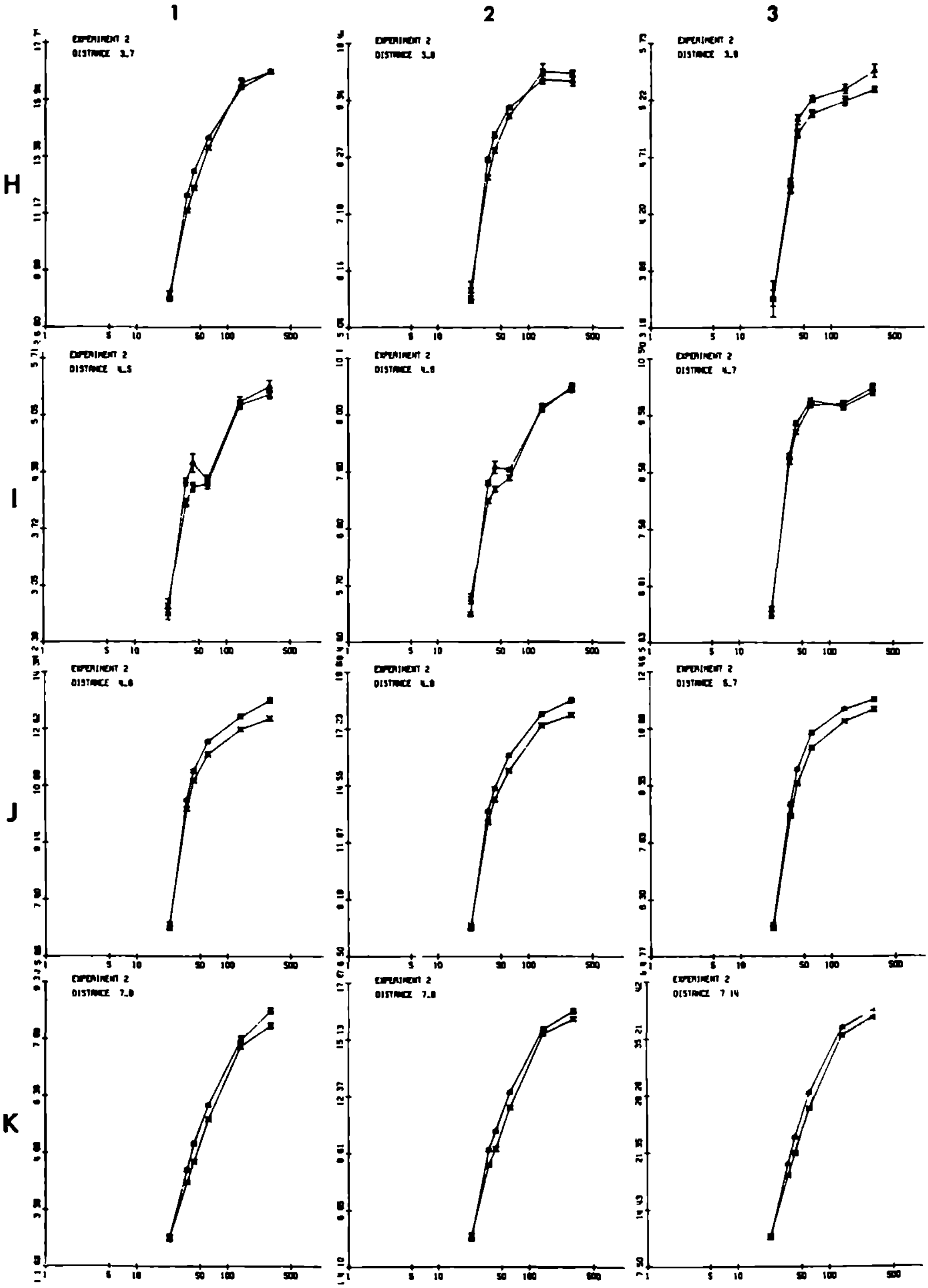
EXPERIMENT 4
DISTANCE 1.6
EXPERIMENT 4
DISTANCE 1.7
EXPERIMENT 4
DISTANCE 2.3
EXPERIMENT 4
DISTANCE 2.5
EXPERIMENT 4
DISTANCE 2.8
EXPERIMENT 4
DISTANCE 2.7
EXPERIMENT 4
DISTANCE 2.10
EXPERIMENT 4
DISTANCE 2.11
EXPERIMENT 4
DISTANCE 3.4
EXPERIMENT 4
DISTANCE 3.5
EXPERIMENT 4
DISTANCE 3.6


1
2
3
H
I
J
K
EXPERIMENT 2
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EXPERIMENT 2
EXPERIMENT 2
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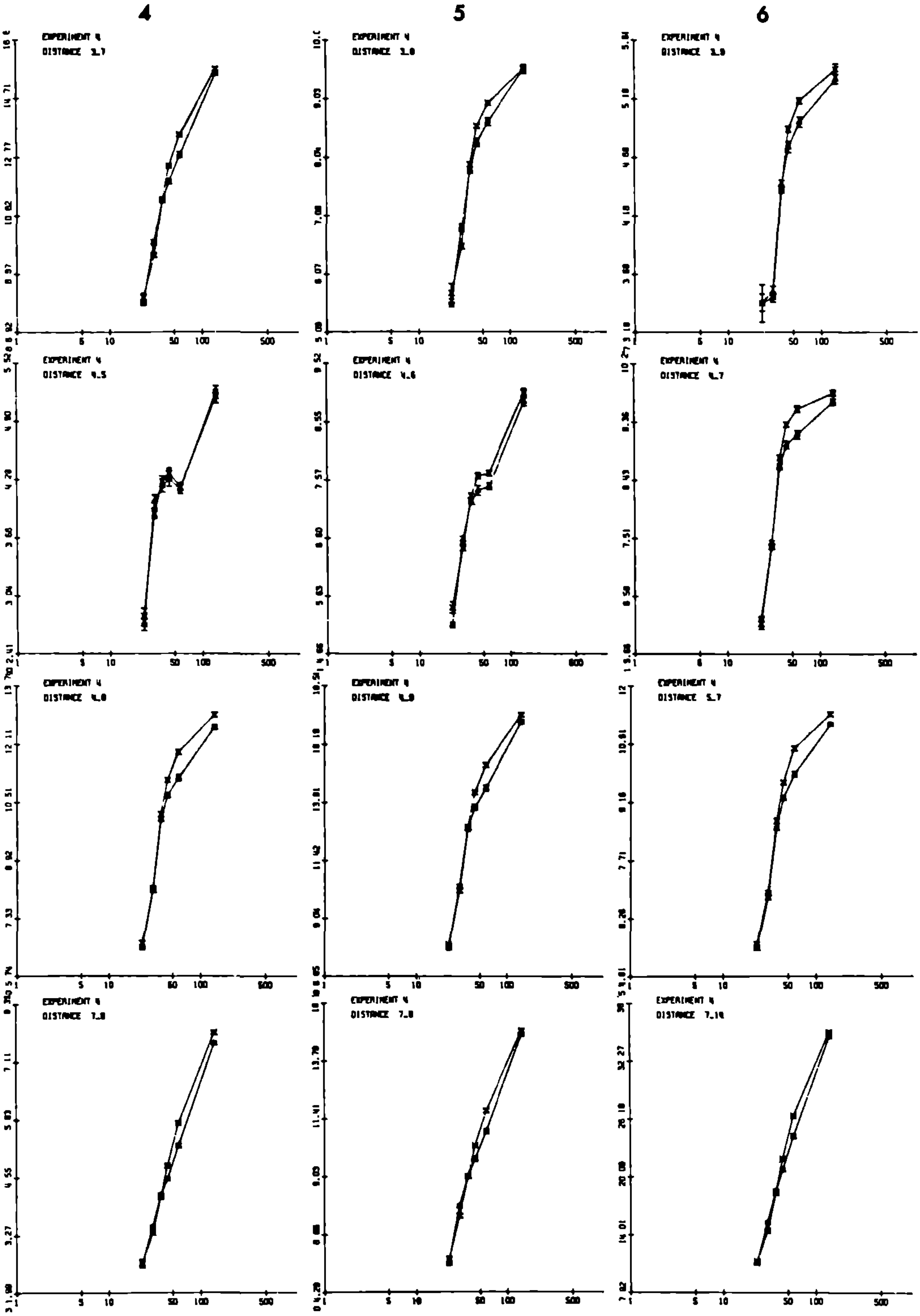
EXPERIMENT 2
EXPERIMENT 2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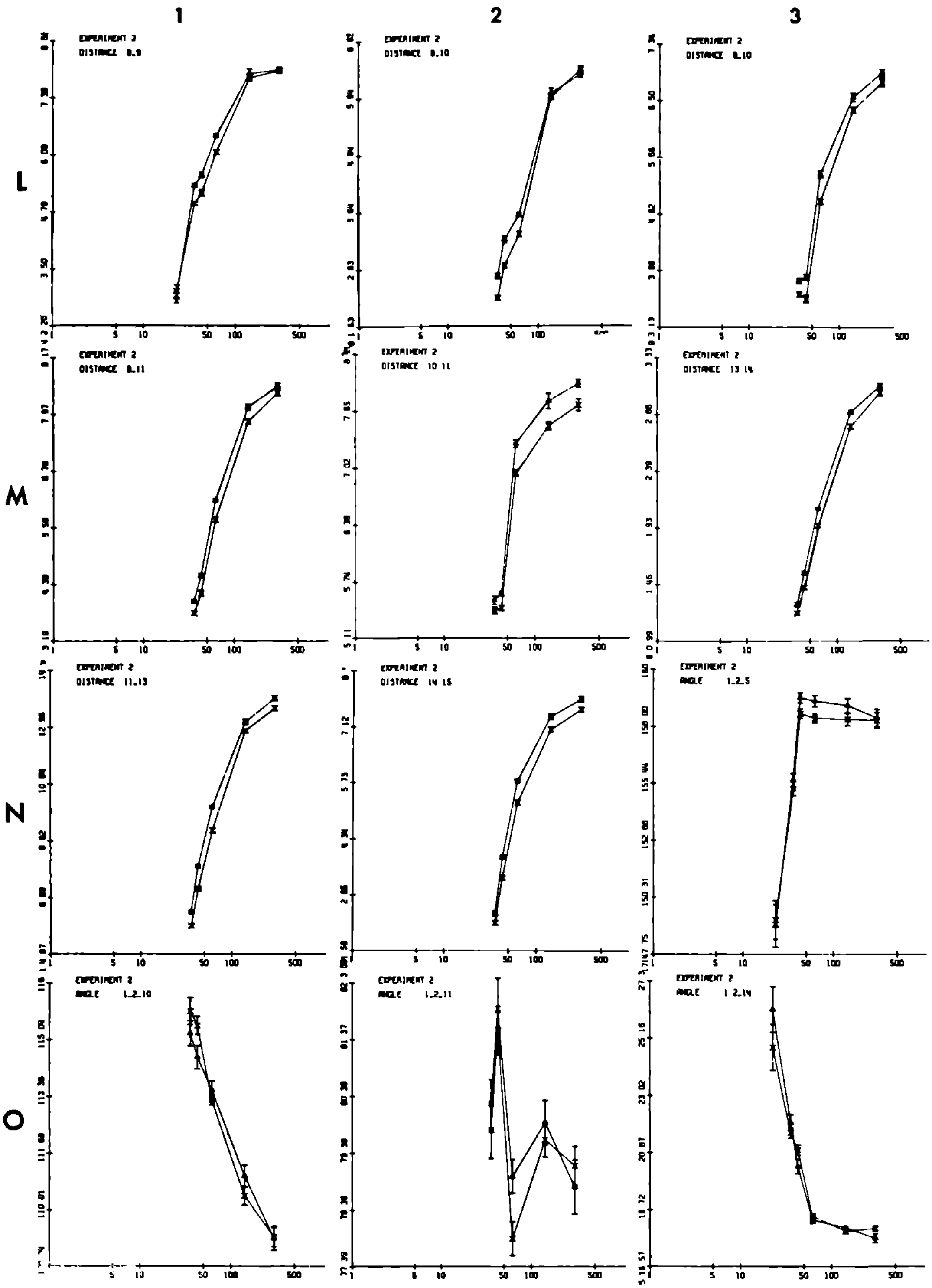
5
6
EXPERIMENT 4


1
2
3
L
M
N
O
EXPERIMENT 2
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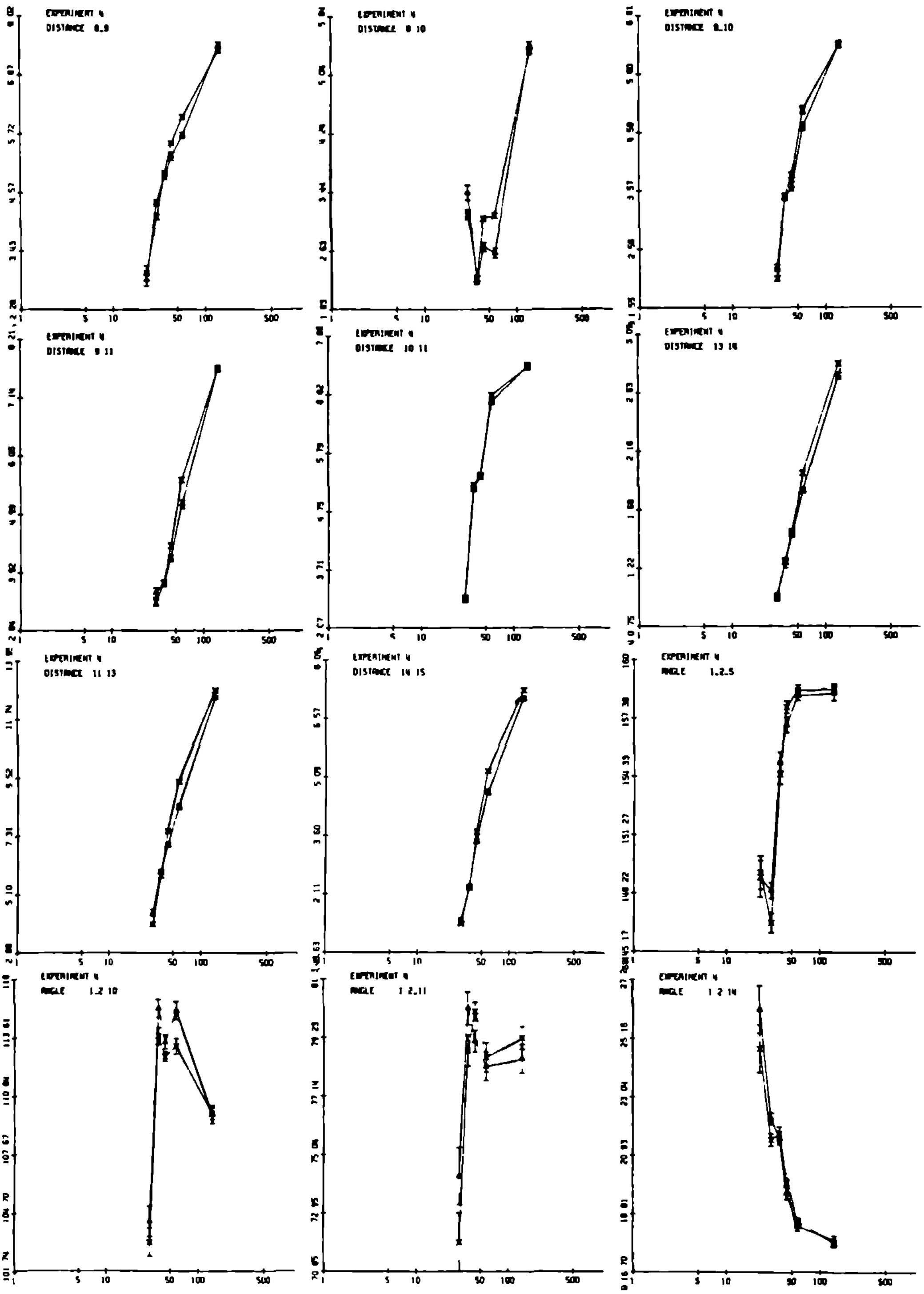
DISTANCE 13 14
DISTANCE 11.13
DISTANCE 14 15
ANGLE 1.2.5
ANGLE 1.2.10
ANGLE 1.2.11
ANGLE 1.2.14

4 5 6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EXPERIMENT 4
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DISTANCE 8.10
EXPERIMENT 4
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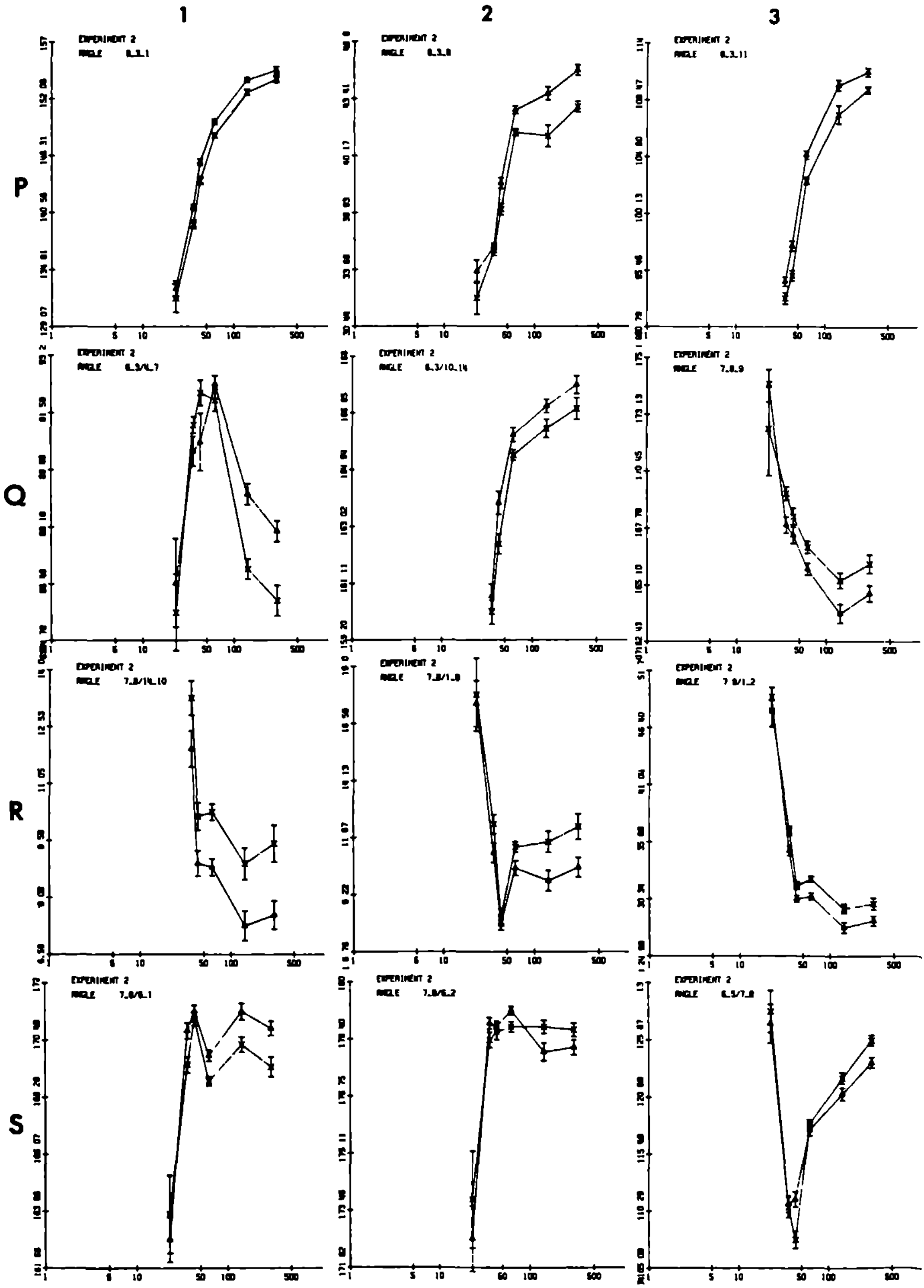
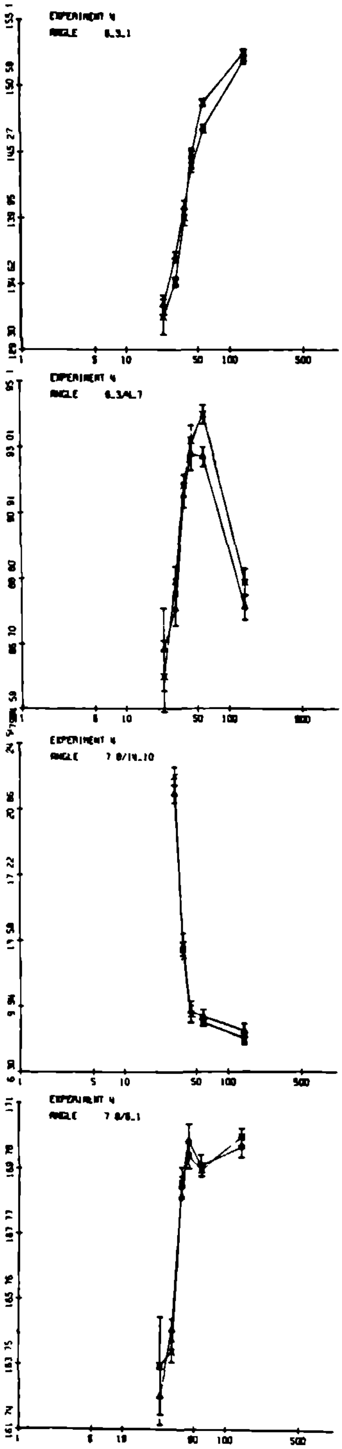
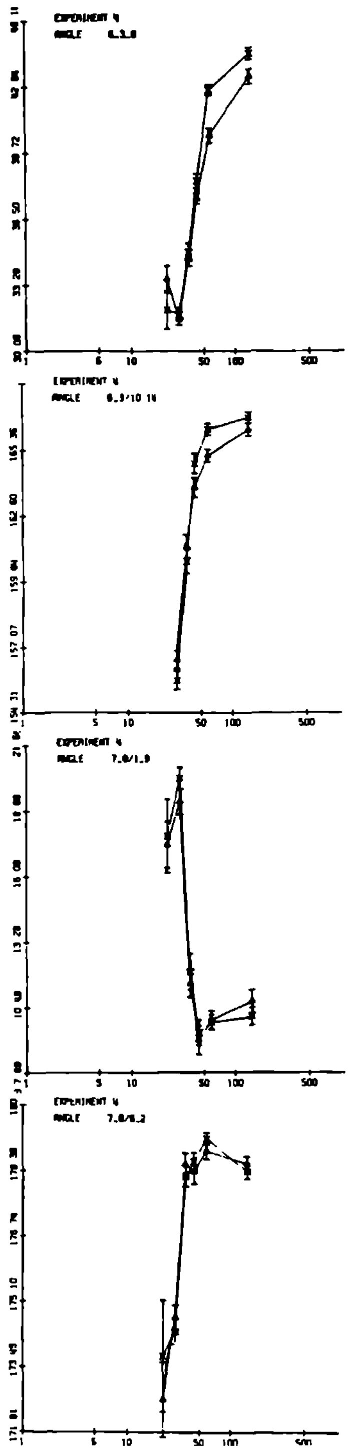
EXPERIMENT 4
DISTANCE 10 11
EXPERIMENT 4
DISTANCE 13 14
EXPERIMENT 4
DISTANCE 11 13
EXPERIMENT 4
DISTANCE 14 15
EXPERIMENT 4
ANGLE 1.2.5
EXPERIMENT 4
ANGLE 1.2 10
EXPERIMENT 4
ANGLE 1 2.11
EXPERIMENT 4
ANGLE 1 2 14


1
2
3
P
Q
R
S
EXPERIMENT 2

4



5



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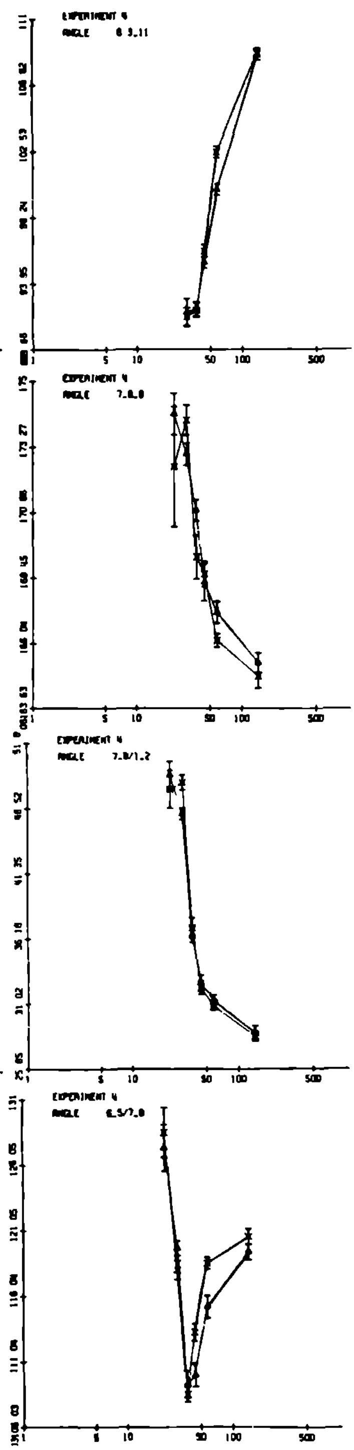
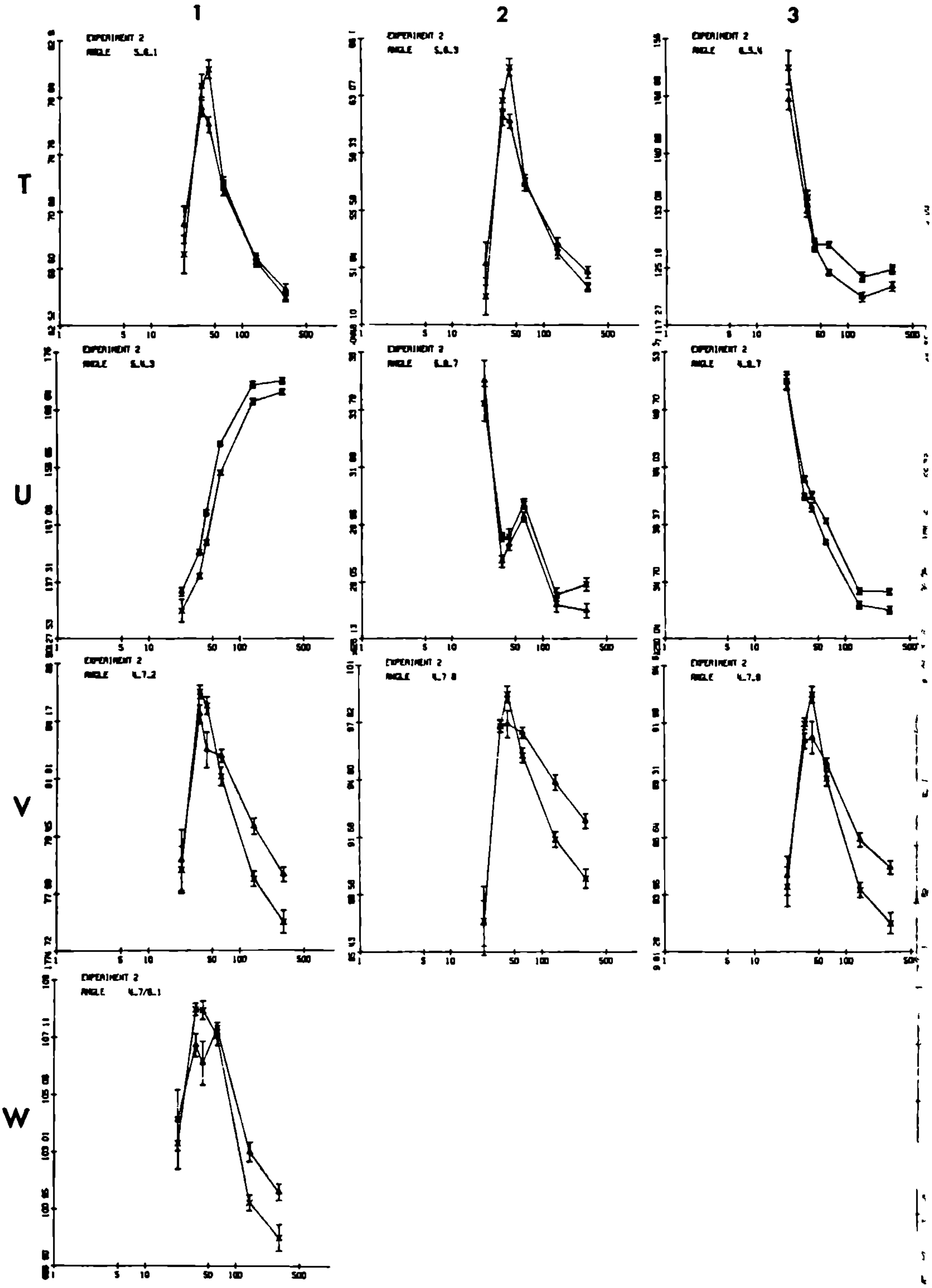
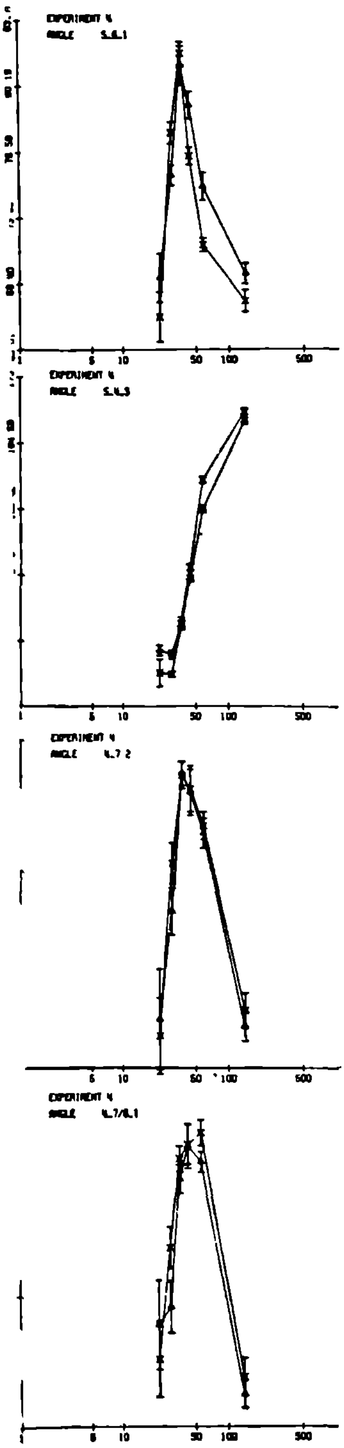
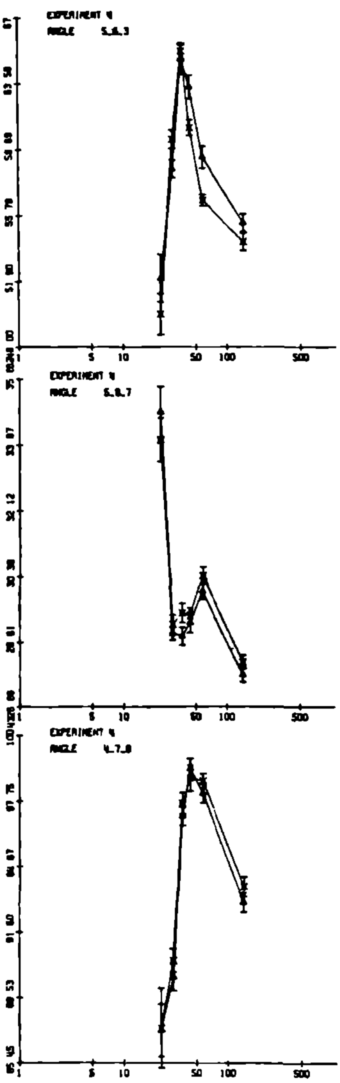



1
2
3
T
U
V
W
EXPERIMENT 2
ANGLE

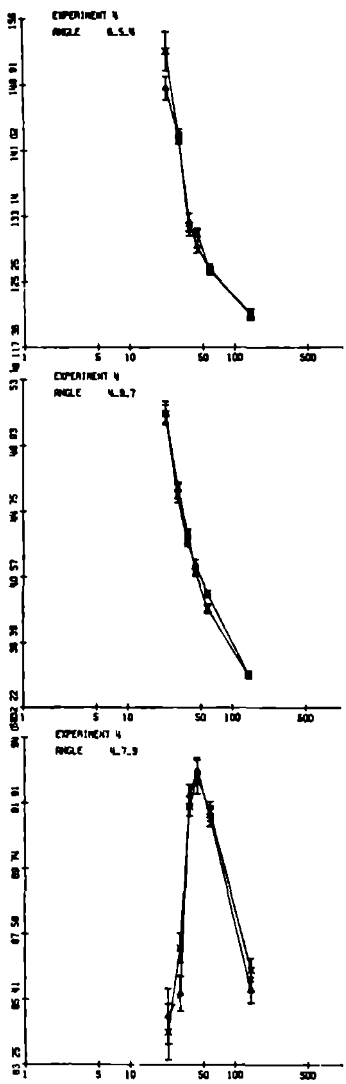
4



5

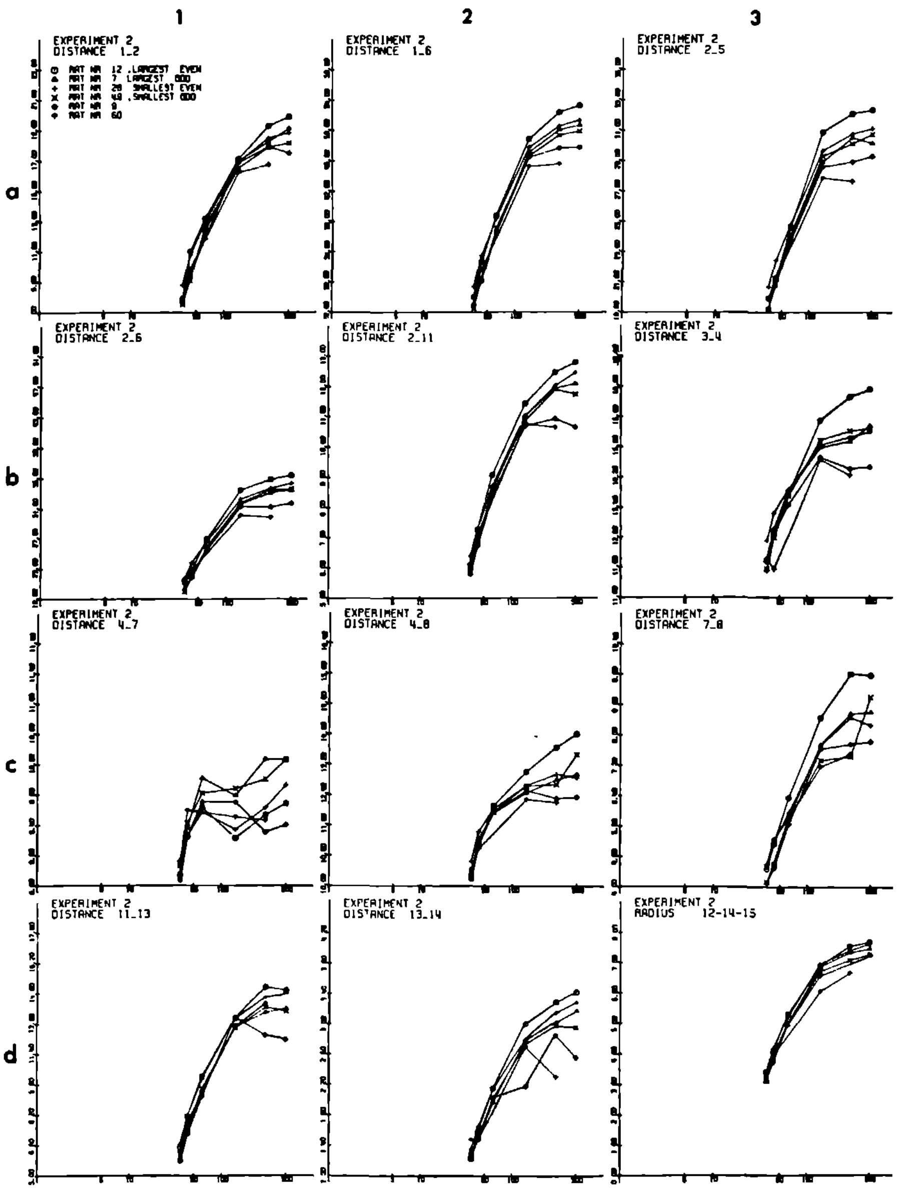


6



PLOTS OF QUANTITIES FOR INDIVIDUALS FROM EXPERIMENTS 2 AND 4

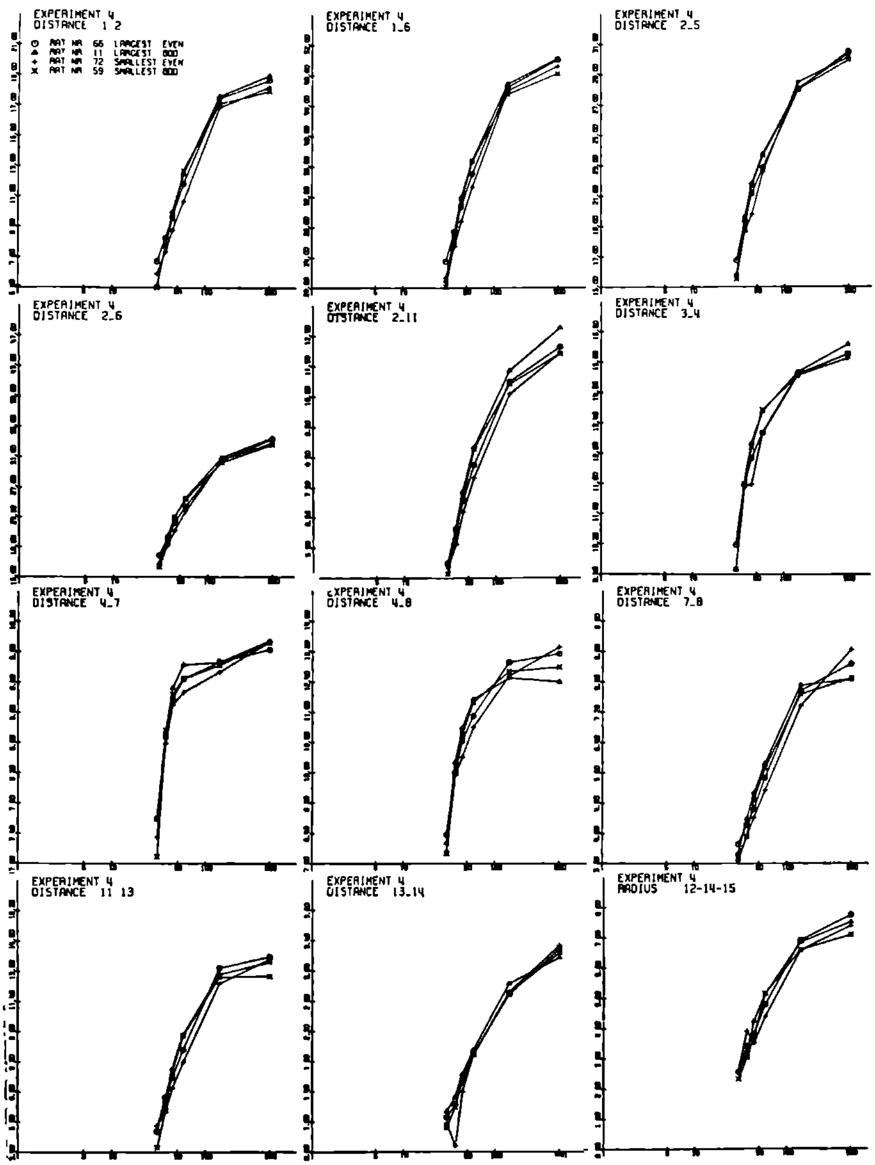
Comparable quantities are placed in corresponding locations on adjacent pages.
The dimensions of distances are given in millimetres, and angles are given in degrees. Areas are in square millimetres. Time is scaled logarithmically for convenience.
These graphs are comparable in scale between the experiments for specific quantities, but

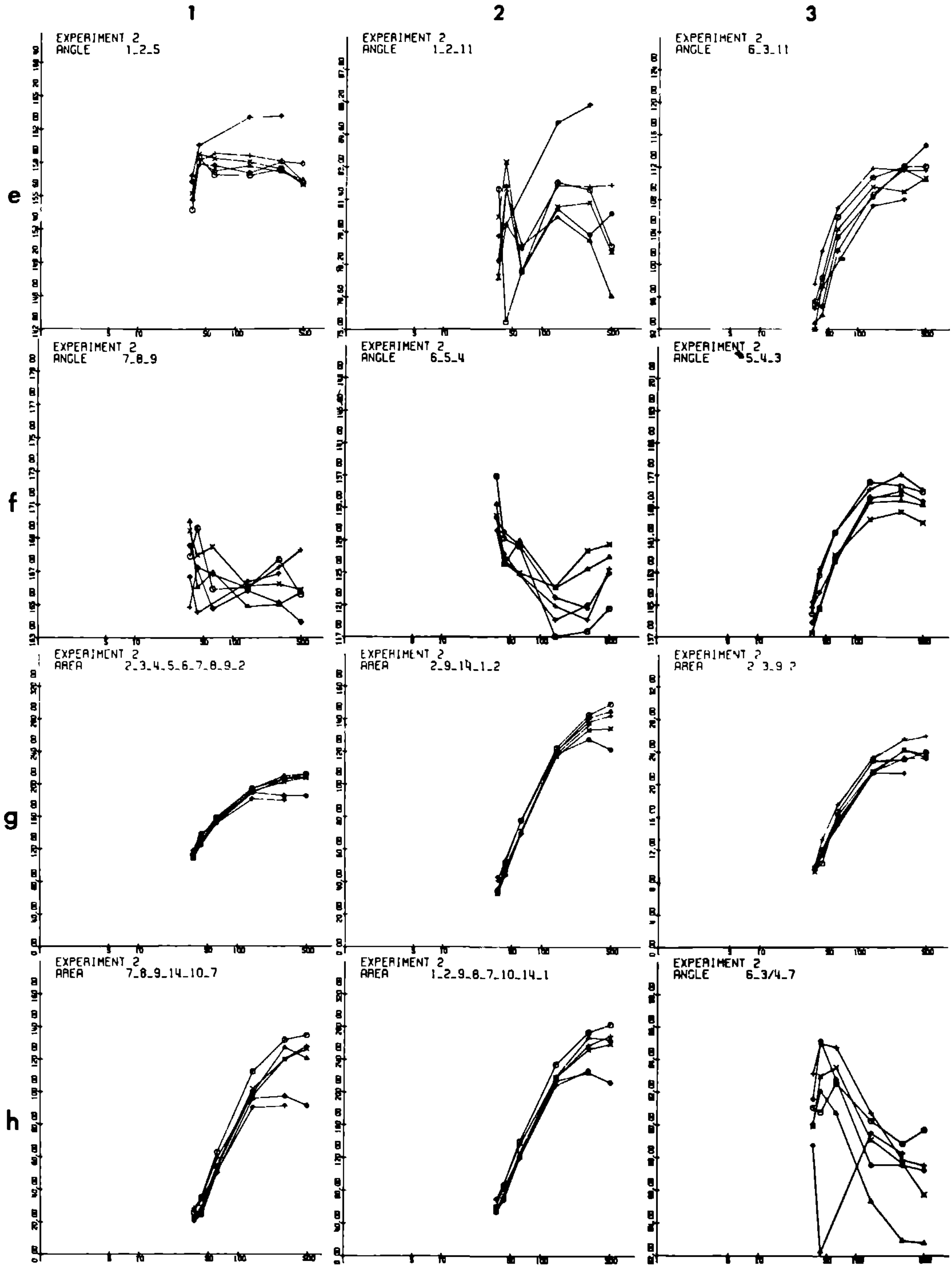


the horizontal axis frequently intersects the vertical axis at different heights in the two experiments. This is due to the attempt to construct the largest possible plots.

It should also be noted that the stages recorded for these individuals do not include stage 1, nor stage 2 in experiment 2. No record was available for rat 60 of experiment 2 in stages 6 or 9, nor for rat 9 of the same experiment in stage 8 involving the incisor radius.

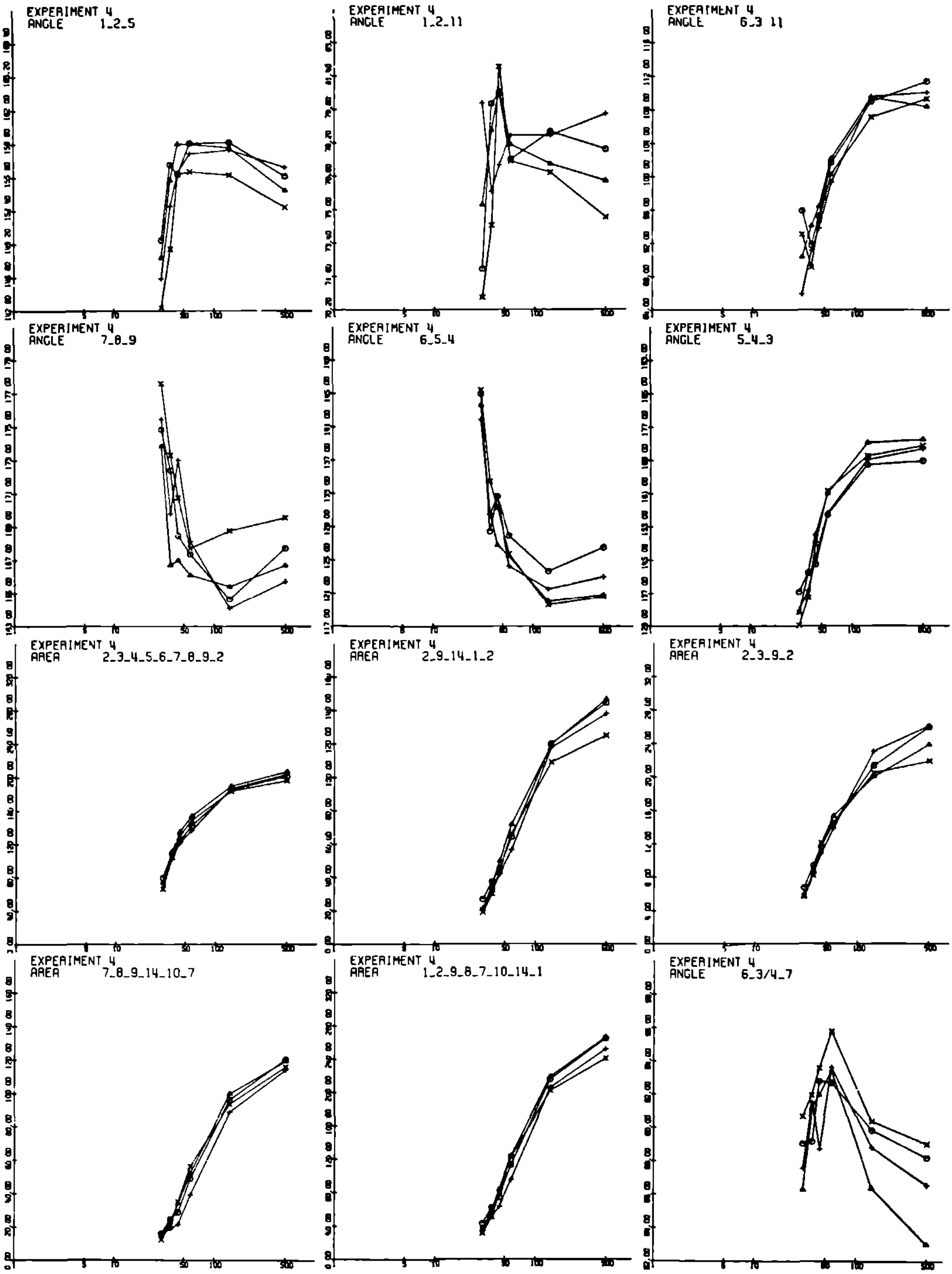
4 5 6



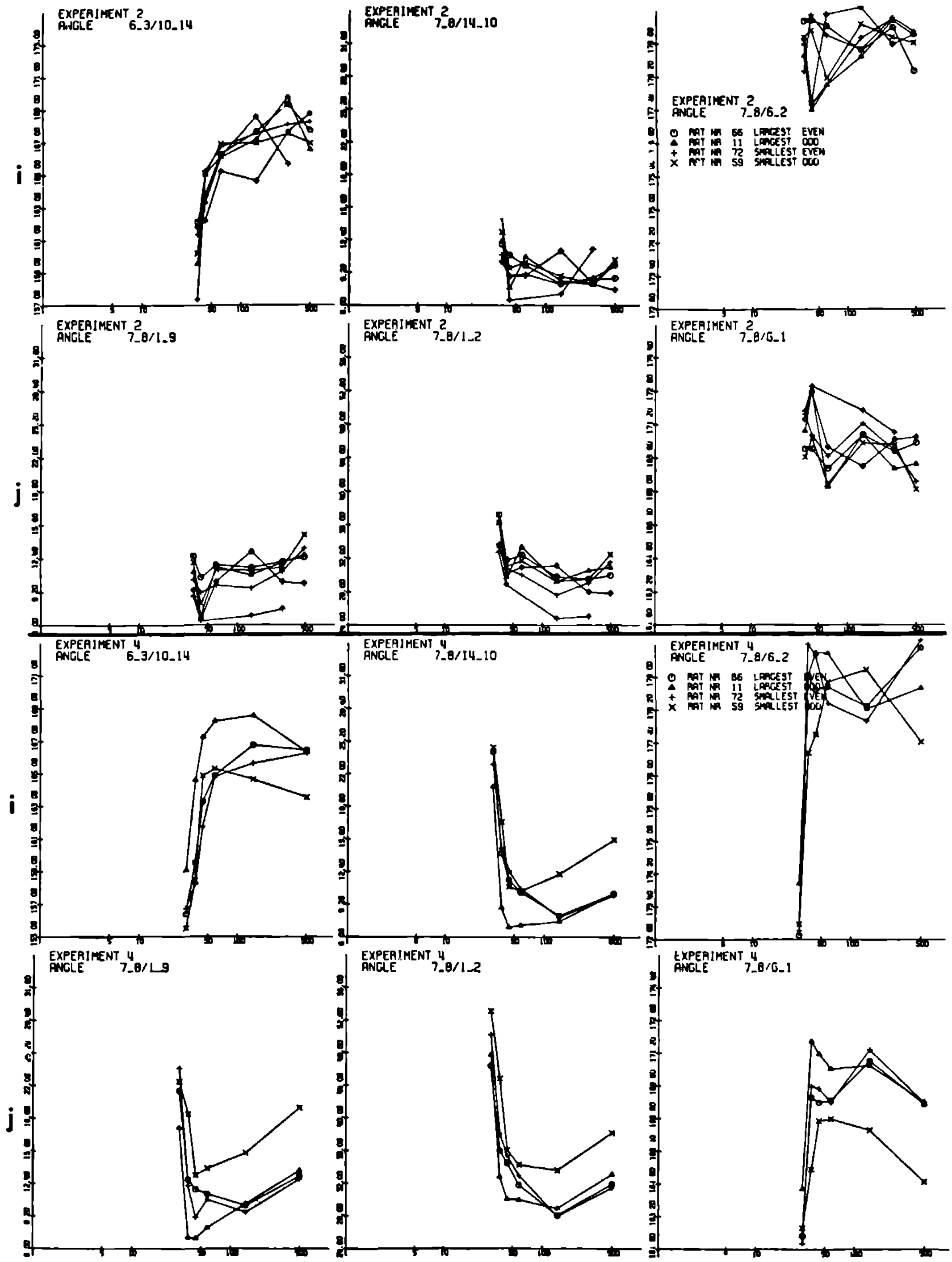


1
2
3
e
f
g
h
EXPERIMENT 2
ANGLE 1_2_5
EXPERIMENT 2
ANGLE 1_2_11
EXPERIMENT 2
ANGLE 6_3_11
EXPERIMENT 2
ANGLE 7_8_9
EXPERIMENT 2
ANGLE 6_5_4
EXPERIMENT 2
ANGLE 5_4_3
EXPERIMENT 2
AREA 2_3_4_5_6_7_8_9_2
EXPERIMENT 2
AREA 2_9_14_1_2
EXPERIMENT 2
AREA 2 3_9 2
EXPERIMENT 2
AREA 7_8_9_14_10_7
EXPERIMENT 2
AREA 1_2_9_8_7_10_14_1
EXPERIMENT 2
ANGLE 6_3/4_7

4 5 6

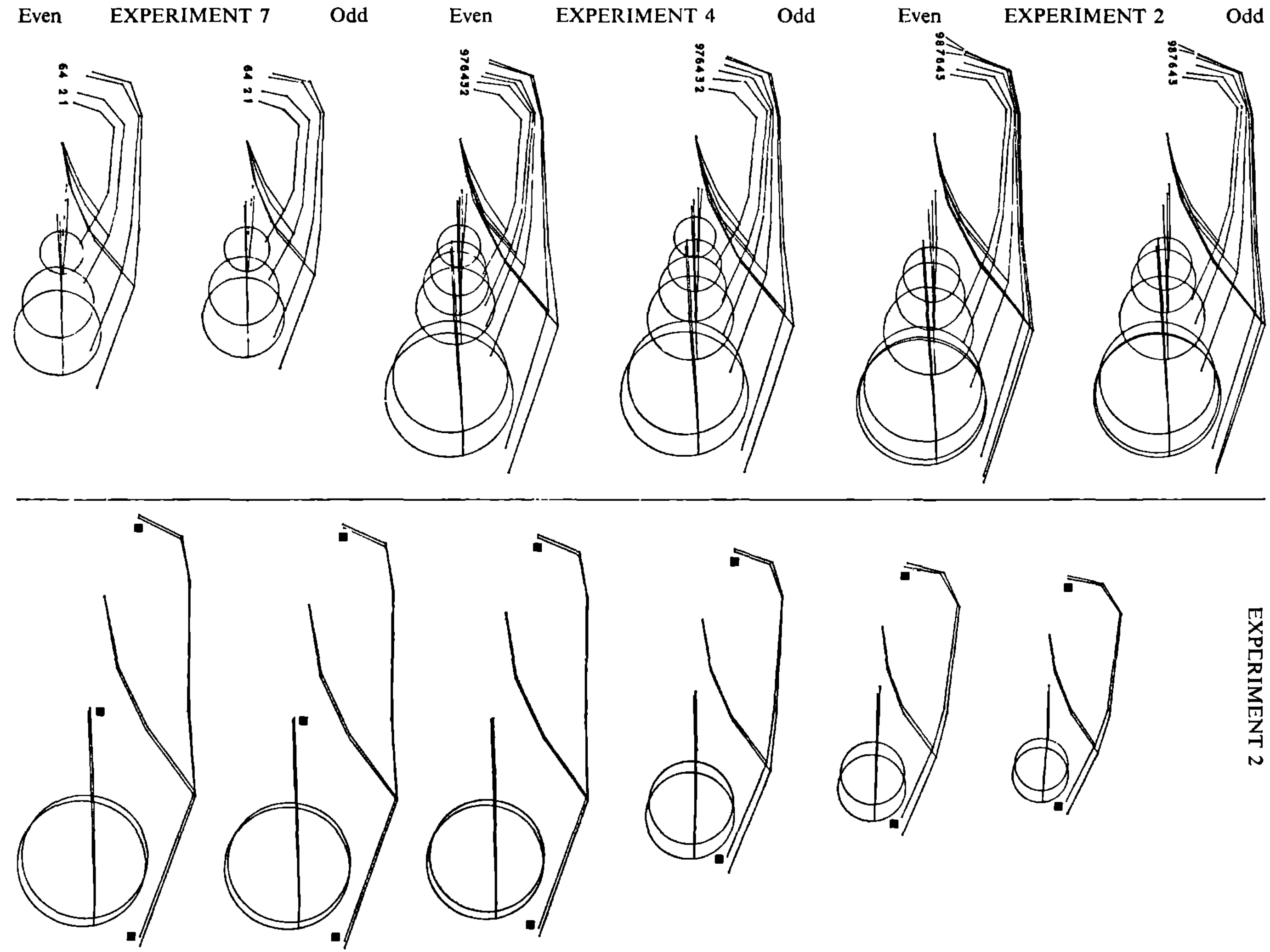

EXPERIMENT 4
ANGLE 1_2_5
EXPERIMENT 4
ANGLE 1_2_11
EXPERIMENT 4
ANGLE 6_3 11
EXPERIMENT 4
ANGLE 7_8_9
EXPERIMENT 4
ANGLE 6_5_4
EXPERIMENT 4
ANGLE 5_4_3
EXPERIMENT 4
AREA 2_3_4_5_6_7_8_9_2
EXPERIMENT 4
AREA 2_9_14_1_2
EXPERIMENT 4
AREA 2_3_9_2
EXPERIMENT 4
AREA 7_8_9_14_10_7
EXPERIMENT 4
AREA 1_2_9_8_7_10_14_1
EXPERIMENT 4
ANGLE 6_3/4_7

1 2 3



EXPERIMENT 2
ANGLE 6_3/10_14
EXPERIMENT 2
ANGLE 7_8/14_10
EXPERIMENT 2
ANGLE 7_8/6_2
O RAT NR 66 LARGEST EVEN
▲ RAT NR 11 LARGEST ODD
+ RAT NR 72 SMALLEST EVEN
X RAT NR 59 SMALLEST ODD
i
EXPERIMENT 2
ANGLE 7_8/1_9
EXPERIMENT 2
ANGLE 7_8/1_2
EXPERIMENT 2
ANGLE 7_8/G_1
j
EXPERIMENT 4
ANGLE 6_3/10_14
EXPERIMENT 4
ANGLE 7_8/14_10
EXPERIMENT 4
ANGLE 7_8/6_2
O RAT NR 66 LARGEST EVEN
▲ RAT NR 11 LARGEST ODD
+ RAT NR 72 SMALLEST EVEN
X RAT NR 59 SMALLEST ODD
i
EXPERIMENT 4
ANGLE 7_8/L_9
EXPERIMENT 4
ANGLE 7_8/1_2
EXPERIMENT 4
ANGLE 7_8/G_1
j
5 10 50 100 500

4 5 6


Even
EXPERIMENT 7
Odd
Even
EXPERIMENT 4
Odd
Even
EXPERIMENT 2
Odd
EXPERIMENT 2

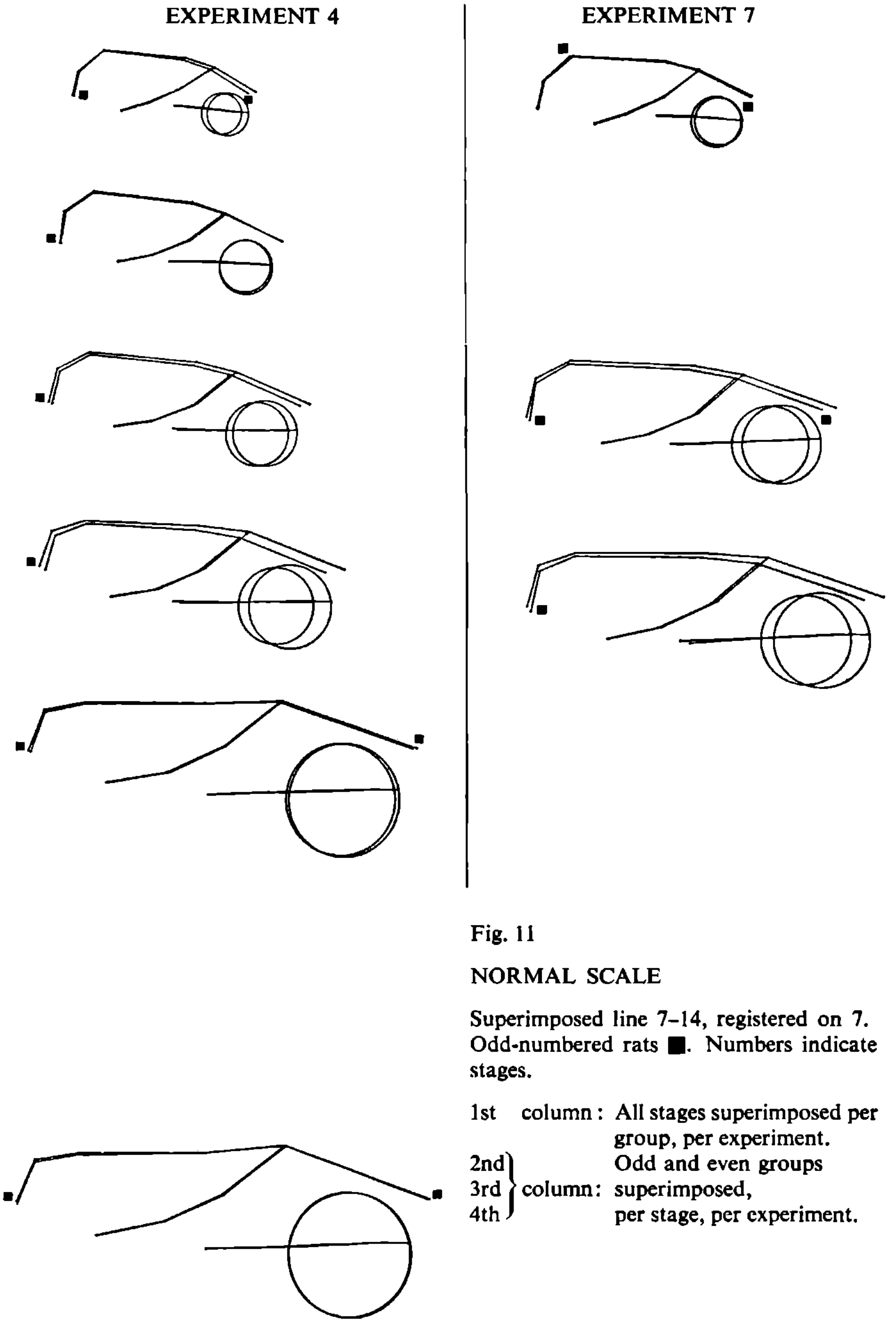


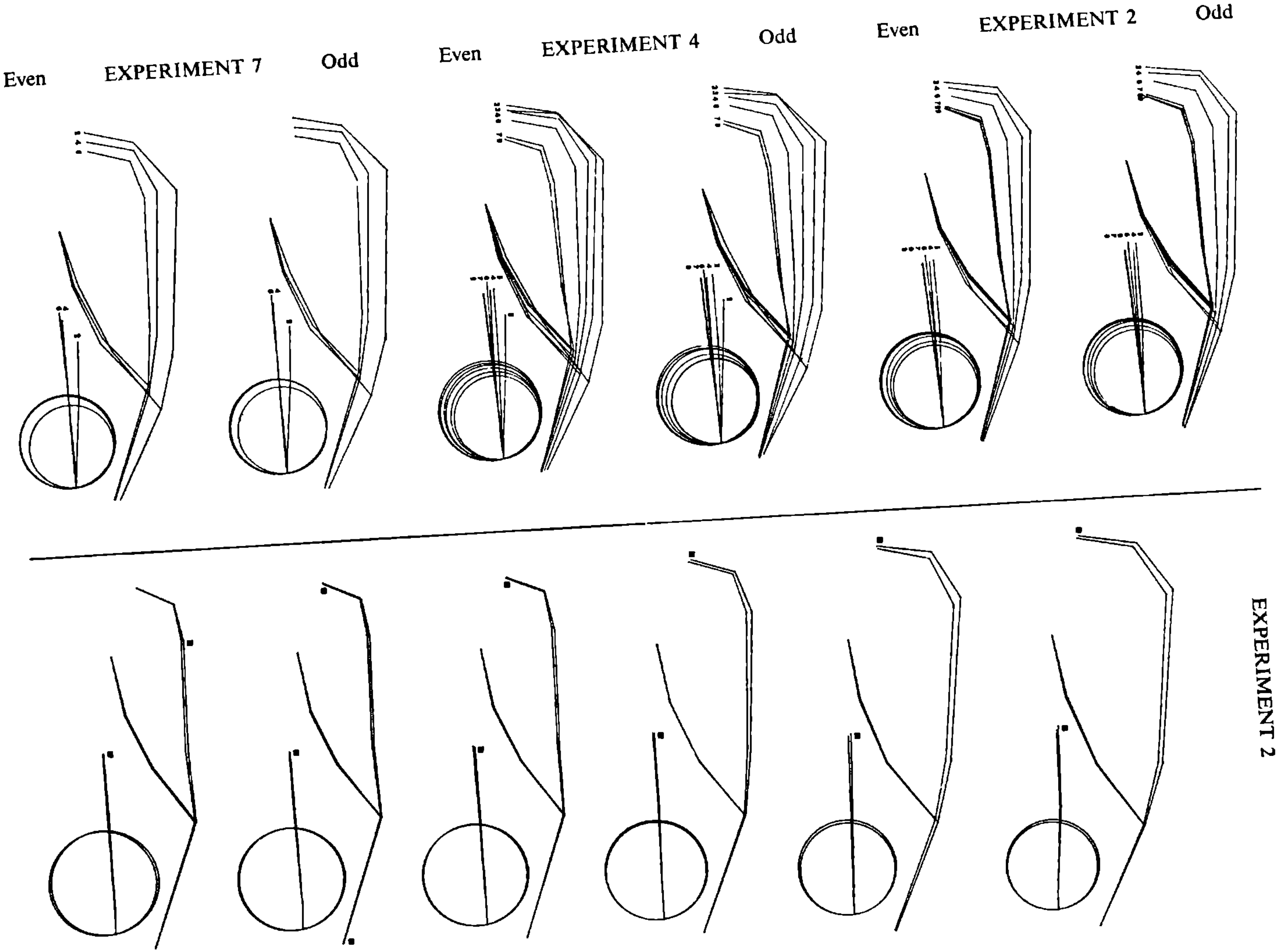
Fig. 11

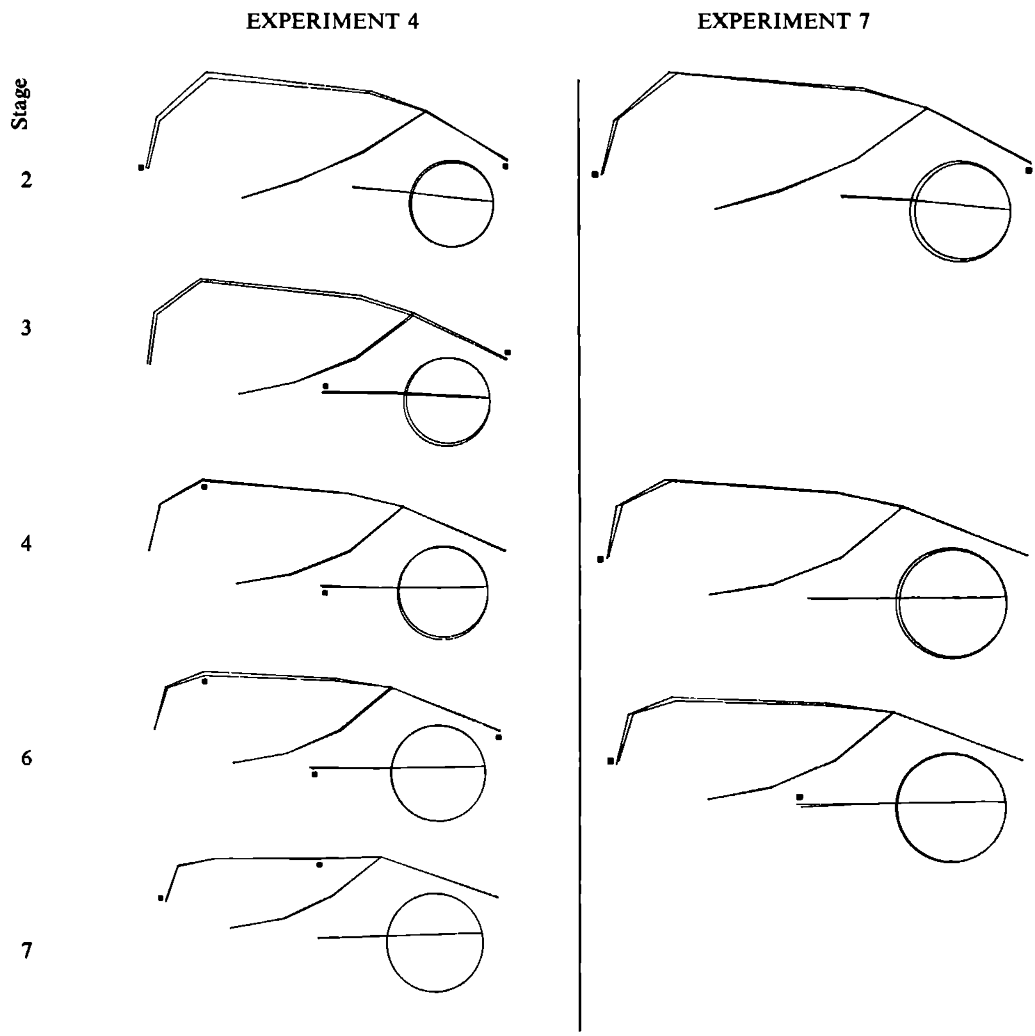
NORMAL SCALE

Superimposed line 7–14, registered on 7. Odd-numbered rats ■. Numbers indicate stages.

1st column: All stages superimposed per group, per experiment.

2nd, 3rd, 4th column: Odd and even groups superimposed, per stage, per experiment.


Even
EXPERIMENT 7
Odd
Even
EXPERIMENT 4
Odd
Even
EXPERIMENT 2
Odd
EXPERIMENT 2



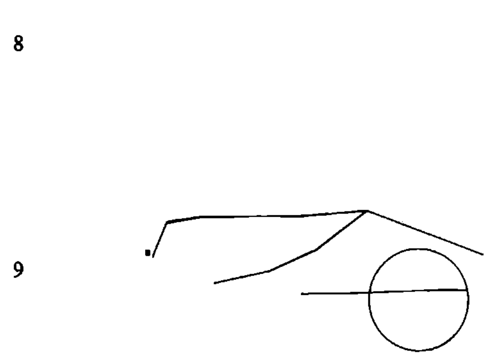


Fig. 12

HORIZONTALLY STANDARDIZED SCALE

Distance 7–14 is held constant. This emphasises change in form.
Superimposed line 7–14, registered on 7. Odd-numbered rats ■. Numbers indicate stages.

1st column: All stages superimposed per group, per experiment.
2nd, 3rd, 4th columns: Odd and even groups superimposed, per stage, per experiment.

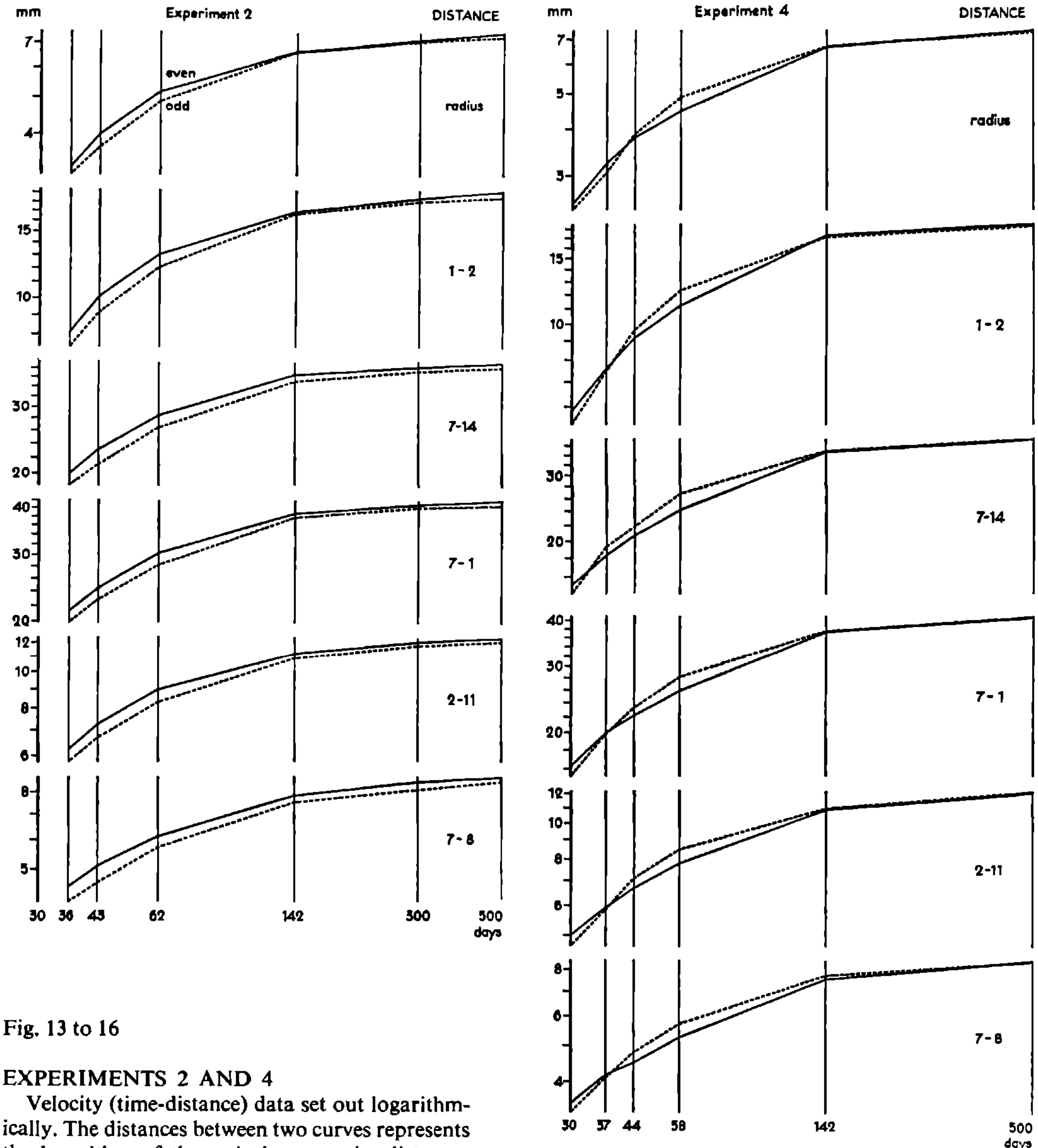
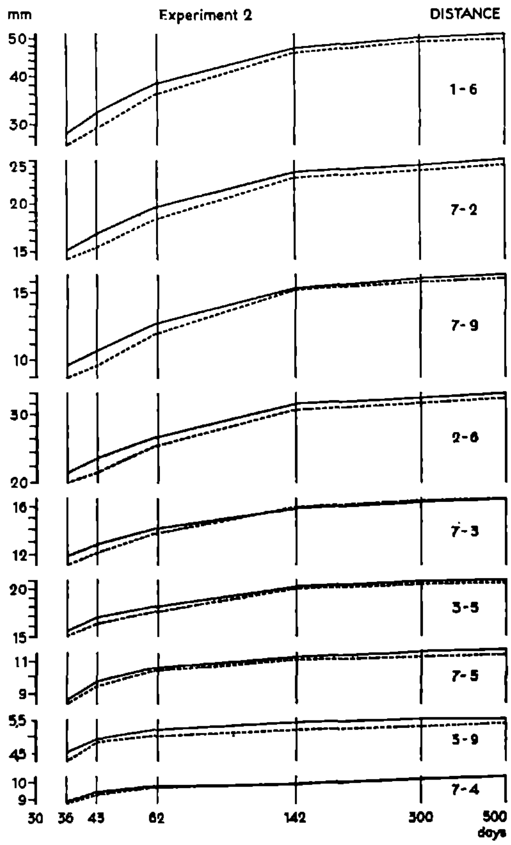
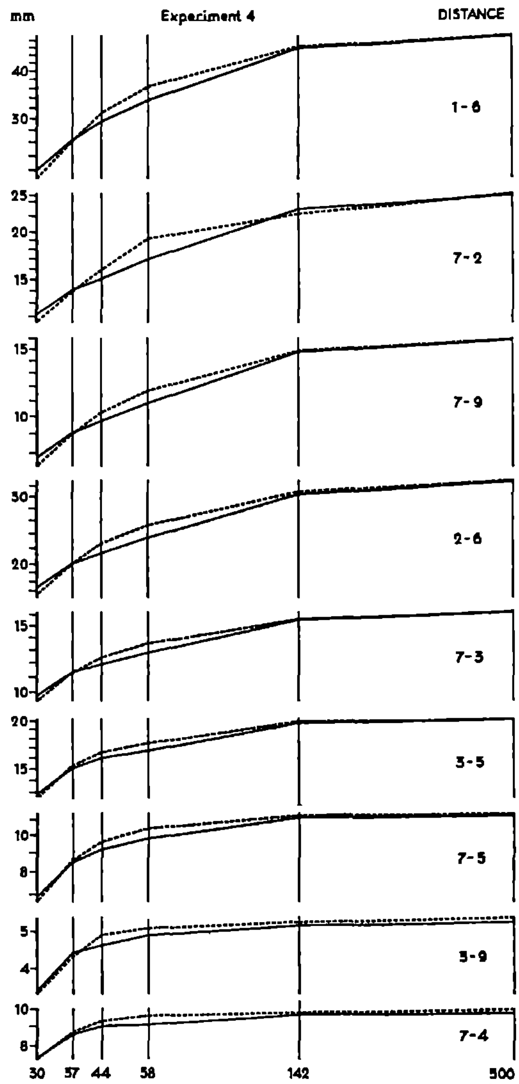


Fig. 13 to 16

EXPERIMENTS 2 AND 4

Velocity (time-distance) data set out logarithmically. The distances between two curves represents the logarithm of the ratio between the distances at that time. The vertical distance between two points on one curve represents the logarithm of the relative increment between the two points in time. When the scale on both axes is the same, as it is here, a slope of 45° indicates no acceleration, above and below 45° indicating acceleration and





deceleration respectively. If two quantities have the same relative increments but different absolute size, their curves will converge at a rate depending on the ratio between the absolute sizes. Here it is clear that compared with experiment 2, experiment 4 has a reversal in behaviour following the change at day 30. The reversal is least rapid in dimension 3 9, and incisor radius. Note how dimensions are ranked according to rates of increase in length.

THESES

1. Study of the head is far more important than study of the skull.

2. Environmental factors can have an effect on the size of teeth.

3. The interparietal bone of the male Wistar rat appears not to grow in length mid-sagittally between post-conception days 30 and 60 and thereafter resumes growth.

4. Growth of bony units of the rat skull is readily discernible after one year of age, although according to Massler and Schour corresponding apposition of bone is not to be found.
   (Massler, M and Schour, I 1951: Anat. Rec., 100:83–101.)

5. No condition in living organisms is static.

6. The reduction of facial development in primates may quite as well be a cause of the assumption of the upright posture as its result.
   (Scott, J. H. 1958: Am. J. Phys. Anthrop., 16:319–348.)

7. Failure to use x-ray pictures for diagnosis in dentistry is a form of professional negligence.

8. The primary responsibility of professional men is to their patients, not to their colleagues.

9. Inadequate attention is paid to training academic staff of universities in teaching methods.

10. The place of mathematics in biological training is generally illconceived.

11. Computers are the source of a flood of information in which many an investigator will drown.

12. There is almost no difference between dog-training and bringing up small children.

Theses appertaining to the dissertation "Growth Pattern and Environment"
by John F. Jefferys, Nymegen, January 1969.